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ACTA RADIOLOGICA

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ACTA RADIOLOGICA
EDITA IUSSU SOCIETATUM RADIOLOGICARUM
DANIÆ, FENNIÆ, NORVEGIÆ ET SUECIÆ

VOL. 41 JANUARY 1954 FASC. 1

CONFERENCE ON RADIOBIOLOGY AND RADIATION PROTECTION

STOCKHOLM

September 15—20, 1952

A conference on radiation biology and radiation protection was held in Stockholm on September 15th to 20th, 1952. Those invited to attend were members of The Joint Committee on Radiobiology, The International Commission on Radiological Protection and The International Commission on Radiological Units, as well as a few selected lecturers and guests.

All the lectures were given on one day, September 16th. The remaining days were taken up by separate meetings of the three commissions and by joint sessions. The following questions, among others, were then discussed, partly against the background of the lectures:

1. What doses per year and per capita of the population are, according to our present knowledge, permissible from a genetic point of view?

2. What radiation doses, under different conditions, cause significant blood changes?

The Editor of Acta Radiologica has kindly permitted reports of the lectures given at the conference, as well as of some of the longer contributions to the discussions, to be assembled in this issue. The undersigned take this opportunity of thanking the Editor in the name of the members of the conference, for the courtesy in this matter.

G. DE HEVESY R. SIEVERT

INAUGURAL SPEECH DELIVERED AT THE OPENING OF THE CONFERENCE

by

Arthur Thomson
Chancellor of the Swedish Universities

Ladies and Gentlemen.

When I was asked to speak at the opening of this conference, I felt that the subject of your meeting was somewhat foreign to me. I therefore turned to our specialists for information.

I soon realized that the problems to be discussed at this conference are not only of great interest to research workers in many branches of science; they are also of vital importance to mankind.

Time has brought many changes.

At the beginning of this century, a radium preparation containing one-tenth of a gram of radium was considered to be an appreciable quantity, worthy of respect. The value of such a quantity then represented a present-day value of more than 100,000 Swedish Crowns.

I am informed that for the same sum it is now possible to obtain a quantity of radiocobalt that emits, over a period of several years, some 10,000 times more gamma radiation than a tenth of a gram of radium.

At the beginning of this century, the highest voltage which could be used in practical work for the production of roentgen rays was about 200,000 volts.

Now, I am told, 20 million volt roentgen rays can be produced without difficulty and, soon, 200 or perhaps even 2,000 million volt rays will be available for practical purposes.

Today, we are also able to produce new types of rays, fast neutrons for example, which have in many substances practically no absorption at all. There are also the slow neutrons, about the biologic effects of which we as yet know very little.

What all this means from a radiation hazard point of view is easy to understand even for a non-physicist. I feel we ought to realize that, because of radiations of various kinds, life may become endangered in manners hitherto unknown. This is all the more evident in view of the increasingly rapid technical application of scientific results.

It is well known that the discovery of roentgen rays and radioactive substances was accompanied by injuries to a number of research workers, doctors and nurses. Unless great care is taken, we may in a near future face a similar situation, but on a much larger scale.

Thanks to the progress in radiologic sciences, we are far better plaecd than the pioneers of fifty years ago. But, the enormous radiation energies which can be released with the new methods involve numerous unforeseen hazards. Some of them may only be recognized when injuries have already occurred.

There is another aspect to be considered. In the near future, machines and substances emitting dangerous radiations will be used for many practical purposes by people with insufficient knowledge of the characteristics and effects of such radiations. This will make radiation hazards a social question of great importance. It will be necessary to take radical measures in order to prevent serious injuries to individuals and future generations.

With this possible picture of the shape of things to come, the present conference assumes the greatest importance. It appears to me that primarily those working in radiophysics and radiobiology are in a position to penetrate the veil that hides the future concerning these matters and form an idea of what radiation hazards the future holds in store for us. These questions already constitute an important part of science.

Your programme illustrates some of the fundamental problems of radiobiology and radiation protection.

The statement of permissible radiation doses, especially from the genetic point of view, forms a central problem, serious in nature and difficult to solve. The measurement of radiation doses in practice is no doubt of great importance and essential for all radiation protection work. The possibility of developing methods for reducing the radiosensitivity of the human body is most interesting.

We have the great pleasure of seeing here a group of outstanding specialists in radiophysics and radiobiology coming from different parts of the world to exchange thoughts and experience.

We are grateful that you have chosen Stockholm as the meeting place for your conference. To all of you I extend a sincere and hearty welcome.

In a field which lies in close proximity to atomic energy research, an attempt to establish international cooperation is, nowadays, a delicate subject especially when small nations are concerned. But such co-operation is a fundamental condition for the sound development of science. International scientific collaboration is a most valuable factor in our efforts to preserve peace throughout the world.

I also believe that the fact that, even in science, national frontiers are at present unavoidable, must be felt as a heavy burden by the scientist. National frontiers are in this case one of the many signs that we live in a sick world.

The present conference will deal with the problem of man's protection against one of the many dangers that threaten him. Its object is the application of science to the benefit of humanity.

I now declare this conference open and express my best wishes for its success.

FROM INDIANA UNIVERSITY, BLOOMINGTON, U. S. A.

THE MANNER OF DEPENDENCE OF THE "PERMISSIBLE DOSE" OF RADIATION ON THE AMOUNT OF GENETIC DAMAGE

by

H. J. Muller

The question of what dose of radiation should be regarded as permissible is one that cannot be answered categorically. It depends in the first place upon the criteria used in forming a judgment in matters of ethics. I will assume that the objective of medical and other practices involving radiation, as well as anything else, is to do whatever will result in the greatest good, or the least harm, when everyone affected by the given action is, so far as possible, taken into consideration. And any injury to health or well-being, physical or mental, is to be regarded as harm.

To decide whether a given irradiation should be carried out, one must know what alternative procedures are possible. This would include knowing, first, what different types of irradiation could be conducted, that is, how the voltage, the filtering, the timing, and the shuttering or limitation of the field may be modified, and also what other methods might be used along with or in place of irradiation. It would include knowing, secondly, to what extent such modifications of irradiation, or substitutions of other methods, are capable of attaining the desired therapeutic, diagnostic or other practical ends; thirdly, knowing the amount of inconvenience and of expense to the operator and to the subject which each such procedure will entail and, fourthly, how much damage, or risk of damage, will result both to the exposed individual himself and to his descendants. The second point, the estimate of the gain for each procedure, must then be balanced against the sum of the third and fourth points, the estimates of the total cost, and that procedure should then be chosen which shows the highest net return, or balance.

Since in most cases only a vague answer can today be given, in any given case, to any of these four ill-bounded and widely ramifying questions, it is evident that present decisions concerning the optimum procedure to be followed in any particular case must to a considerable extent be arbitrary. Even more arbitrary is the setting up of a universal permissible dose, where in fact no sharp line exists. A great deal of intuition must enter into the estimation of any such generalization. Nevertheless, I think it is probably useful to have such a ceiling, just as it is useful to have a speed limit in driving. In arriving at it there are known principles to be taken into account, and quantitative information which must not be neglected does exist along certain relevant lines.

It is up to the specialists in the applications of radiation to decide the first, second and third points, that is, what modifications of their procedure, and what alternative procedures, are feasible, and what the relative value and immediate cost of these different methods may be. Here we are concerned primarily with the fourth point: the amount of damage which a given amount of radiation may result in [illegible] that this may be balanced against the benefit. Moreover, in considering the damage, we will here give attention only to the genetic damage appearing in later generations. This limitation is set not only because that aspect happens to be the particular field of investigation of the present author, but also because, in cases of whole-body irradiation, as well as in those of irradiation of the pelvic region, and even, sometimes, in irradiation of some other regions, when the pelvic region is not well protected from outlying and scattered radiation, this genetic damage to posterity is probably greater, in its totality, than that usually caused to the exposed individual himself. This conclusion is arrived at by comparing the data for the shortening of life in mammals, and for cancer and leukemia, associated with a given amount of radiation, with the data on genetic effects. For this reason the entries on the damage side of the ledger, in the case of exposures of this kind, should be based chiefly on the extent of the genetic effects. However, it will be desirable, in future more definitive surveys, to have the somatic effects on the exposed individual added in, especially in those cases where special parts of the body, other than the pelvic ones, have constituted the main field of exposure.

A primary point, in the consideration of the genetic damage to later generations, is the fact that each mutant gene received by an offspring will on the average constitute a deciding cause of what may be called the "genetic death" of one descendant — that is, of his dying before reproduction, or failing to reproduce. This does not mean that it is a "lethal" gene in the technical sense of causing so gross a morphological or physiological abnormality as to kill before maturity every individual in whom it finds expression. Most mutant genes are not nearly so drastic

as that. Even when homozygous (received from both parents alike), they cause but a relatively small amount of impairment, which is commonly not recognized or may be quite imperceptible by ordinary means of observation.

Modern work indicates that few mutant genes are completely recessive, and that when heterozygous (received from only one parent, with the other parent furnishing the normal alternative gene) they produce something like a twentieth or a thirtieth as much effect as when homozygous — the proportion varying greatly according to the gene under consideration, and according to accompanying environmental conditions and other genes. At least, that is the situation in *Drosophila*, as studies both of STERN and NOVITSKI (1948, 1952) and of my own group (1950) show, and there are good reasons to conclude that a similar situation exists in man also, as work of LEVIT (1936) has indicated. Yet even the small amount of damage done by the gene in its heterozygous condition is usually significant in the long run. It can be shown that it will in consequence be a determining cause of the genetic death of, on the average, one descendant carrying the given mutant gene in heterozygous condition, before it has had a chance to become homozygous, or even, having become homozygous, after it has again become heterozygous.

Ordinarily this will happen many generations after the mutant gene has arisen by mutation. In the meantime the gene will have passed on down through a long succession of heterozygous individuals, whom it has hampered but slightly, without their having been put by it into a definitely abnormal category. We all of us carry a fairly high number of such genes, which collectively hamper us and constitute what may bi-called our genetic "load", and each of which must eventually be eliminated by becoming, some time, a deciding cause of genetic death on the part of some descendant, usually a remote one. The total vigor, resistance and general fitness of each person, apart from environmentally determined differences, depends on the combined action of all these genes of his.

Now it might be supposed that any one mutant gene having an extremely small effect would be practically negligible, and could at any rate be compensated for by intelligent hygienic and medical practices, by the betterment of living conditions, etc. However, the smaller a gene's damaging effect is, the more generations it passes through before causing genetic death, precisely in inverse ratio, and so its total contribution to the loss of fitness or genetic load of the future population is, in the end, the same as that of a major "lethal" gene. It will eventually be a cause of one genetic death, as surely as a real lethal is, and it may be regarded as contributing, all told, the equivalent of one unit of load to the fitness of the population. The improvement of living conditions and of medical

care will decrease its effect on the single individuals who carry it, thus dragging out its existence and increasing the number of individuals afflicted with it, without diminishing the final total amount of damage which it does.

However, it is probable that the genetic death of an individual is often caused by the combined action of two or three slightly detrimental genes acting together, commonly too with the cooperation of exceptionally adverse environmental circumstances. For this reason the actual number of individuals who suffer genetic death, in the sense defined above, may be only a half or a third as many as the number of mutant genes which these individuals contain in excess of the average number carried by the population. For certain statistical reasons, which it would take too long a digression to go into here, the average number in excess, carried by the eliminated individuals, could hardly be much greater than three. To be conservative, then, it will here be assumed that the number of excess genes eliminated is three per eliminated individual, and that there are therefore only one third as many genetic deaths, per generation, as there are mutant genes to be eliminated. This many genetic deaths, however, there must be. Otherwise the number of mutant genes in the population would continually increase, as more and more were added in each generation by mutation than were eliminated by genetic death, until the load became impossibly large and the population became extinct. There is, therefore, a kind of equilibrium finally established in any population, at which as many genes are being eliminated in each generation as are simultaneously arising by mutation. And this requires that at least a third as high a frequency of individuals dies by genetic death as the frequency of mutant genes arising.

Let us now examine what this frequency of mutations amounts to. Modern studies on human mutation rates, carried out by HALDANE, PENROSE, DAHLBERG, KEMP, NEEL and their associates, as well as by some others, have joined in making it probable that for most genes in man there is one spontaneous mutation in something like 50,000 gametes, or perhaps in even fewer. This is a good deal higher than the frequency of spontaneous mutations per individual gene in *Drosophila*. And while in *Drosophila* the evidence indicates the existence of five to ten thousand genes per set, more likely the latter, it seems reasonable, on several grounds, to assume that in man there are several times this number. Let us, however, to be conservative, assume that in man there are only one and a half times as many, that is, 15,000 genes. In that case there would in man be 15,000 times 1 in 50,000 new mutations, or 0.3, present in human gametes. That is, three out of every ten sperm or eggs would contain, in some gene or other, a mutation which had arisen in the immediate parental generation, in addition to all the mutations inherited from the more

distant past. This compares with an estimated over-all frequency of spontaneous mutations in *Drosophila* of about 1 in 20 (or possibly 1 in somewhat less than 20) gametes. Some students of the subject are inclined to think that the human frequency is much higher than 3 in 10, because of the existence of many more genes, and possibly too of a higher average mutation rate per gene. Thus NEEL (personal communication) thinks 1 per germ cell likelier. On the whole, then, it appears conservative to adopt for present working purposes the figure of 3 in 10 for human gametes, and this means that among the *zygotes* formed there are at least 6 new mutations in every 10.

Consequently, to maintain an equilibrium, 6 mutant genes must also be eliminated per each ten individuals in each generation. And if, to be again conservative, we assume that it usually takes 3 mutations in excess of the average number present, to cause genetic death by their synergistic action, then the 6 mutations eliminated would be carried by 2 individuals, each having about 3 of them, and so two individuals in every ten would in every generation suffer genetic death, *i. e.* death before maturity or failure to reproduce, or a correspondingly larger number would reproduce insufficiently for genetic maintenance. The remainder, then, would have to multiply enough more than their maintenance amount to compensate for this, making for them an average of 2.5 children per person (couple) if the population is to remain stable in numbers, without making any allowance whatever for premature death or failure to reproduce caused by any other circumstances than genetic ones.

The above is the situation, as nearly as it can now be reckoned on the most conservative premises, so far as the frequency of spontaneous mutations is concerned. To what extent is this altered by the introduction of mutations produced by radiation? Till recently this question was one of the greatest uncertainty on the quantitative side, since there was little to base an estimate on except the results from *Drosophila*, supported by some extremely meagre, barely comparable, gleanings from mice obtained by PAULA HERTWIG, SNELL, and CHARLES. Now however RUSSELL's (1952) precise and extensive results on mutations produced by roentgen rays in 7 specific genes of mice have demonstrated that for 6 of the 7 genes studied mutations are produced in spermatogonial cells with an average frequency of about 15×10^{-5} per r unit per gene, while if the most mutable of the 7 genes is included also, the average frequency is about 26×10^{-5} per r unit per gene. As the latter figure is about seven times as high as the corresponding one for genes in spermatogonia of *Drosophila*, it is probable that the earlier estimates of induced mutation rate in man, based on *Drosophila* results, are not far from one order of magnitude too low, instead of being too high as some critics had suspected. The mouse, to be sure, is not so very close to man, yet considering

the inordinately greater taxonomic distance from *Drosophila* to the mouse than from the mouse to man, and the fact that there is a 5 to 10 times rise in the induced mutation rate per gene when the distance from *Drosophila* to the mouse is traversed, it hardly seems likely that there would be anything like as great a fall as this in passing from the mouse to the human. That is, it is necessary, provisionally, for questions concerning human safety, to use the mouse data as representing the nearest approximation we have to the effects in humans.

The induced mutation frequency of 26×10^{-5} per r per gene may be restated in the form, 1 mutation per r in a given gene among about 4,000,000 gametes. Multiplying this expression by 80, we find that 80 r would produce 1 mutation in a given gene in some 50,000 gametes. But 1 in 50,000 is, as we have seen, the approximate frequency of spontaneous mutations per individual gene in man. That is, 80 r would give a mutation rate equal to the spontaneous one. If, now, there are enough genes for the existing rate of *spontaneous* mutation per gene to sum up to an over-all frequency of at least 3 mutations among 10 gametes, when all genes together are considered, as has previously been inferred, then the application of 80 r of roentgen or gamma rays would, likewise, result in an induced mutation frequency of at least 3 mutations among 10 gametes, and these 3 induced mutations would be added to the 3 spontaneous ones, so that the total mutation frequency would be doubled. There would in consequence be 12 instead of 6 new mutations received by every 10 zygotes, that is, individuals. And if these 12 mutations were usually eliminated by genetic death as efficiently as 3 per eliminated individual, as was assumed in order to be "on the side of caution", then 4 out of every 10 individuals, instead of only 2, would have to suffer genetic death for the maintenance of genetic equilibrium, in a population in which this amount of radiation was the average received per individual generation after generation. The remaining individuals in that case, in order to restore the number 10, would have to produce $3^1/_3$ children per couple, in addition to those compensating for death or sterility due to nongenetic circumstances. At the same time, even the surviving individuals would carry twice as heavy a genetic load, that is, they would be twice as much afflicted by nature as they would have been if they had inherited only spontaneous mutations.

These calculations show us that, considering the slow natural rate of human reproduction, and the large proportion of deaths which under primitive conditions are due to infection, accident, violence, exposure, malnutrition and other largely nongenetic causes, the spontaneous frequency of mutations in man must even under primitive conditions be near that critical level which, if exceeded, would result in a rate of elimination too great to be compensated for by reproduction, and would

therefore cause a continued decline of population until extinction. Under modern conditions, in nearly all technically advanced countries, even though so much of the nongenetic death has been overcome, the rate of reproduction has been artificially decreased to such an extent that we must be still nearer the critical level, or have very likely exceeded it. In this case however there must at present and for some time to come be an artificially reduced rate of elimination also, leading to a decline in genetic quality rather than in population quantity. That is, mutations must at present be accumulating faster than they are eliminated since they are being saved for a longer time from genetic death. And although a new equilibrium would doubtless be reached by the population eventually, after a very long time, this would be established at a level at which there was a much heavier load of mutant genes per individual than at present — enough so in fact to compensate for the improved conditions of living which had allowed them to accumulate.

Under these modern circumstances, then, a doubling of the mutation rate caused by the exposure of generation after generation to 80 r would cause the genetic decline to be twice as rapid, and the equilibrium, if established, would entail twice as heavy a genetic load as in a population not subject to this exposure. However, unless reproductive practices were very different from the present ones and returned to a high fecundity and high elimination rate, either through "natural" causes or by design, equilibrium could not be established with as high a mutation rate as here assumed, and the population size would then inevitably diminish to extinction, despite the long postponement of this process. Of course it is not to be expected that present practices will in fact persist long enough for all this to come to pass, yet any part of it is in itself an evil, in approximate proportion to its duration.

It would be very difficult to estimate, even with extensive anthropological data, just how much of an increase in mutation rate a primitive population could tolerate, without exceeding the critical level beyond which equilibrium is impossible. However, the above considerations indicate that a doubling of the rate, such as might be brought about by 80 r, would probably be too much. On the other hand an increase of some $12\frac{1}{2}$ % in the mutation rate, such as might occur with the exposure of each generation to some 10 r beyond the usual amount, could probably be tolerated with relative ease by a population which has a rather primitive birth and death rate. This is indicated by the fact that the human populations on the high plateaus of Bolivia and Tibet, where the cosmic radiation is probably strong enough to give them an additional 5 r per reproductive generation, appear to get along fairly well genetically (though an exact study of them would be of much interest), despite the fact that they have been living at a very high altitude for so many gener-

ations as to show a number of genetic adaptations to it, such as a naturally large chest. In view of this object lesson on the one hand, and the obvious danger of a doubled rate on the other hand, I would suggest that, provisionally, a 25 % increase in the mutation rate, such as might be brought about by 20 r, be considered as approaching the danger level for the species, for populations living under relatively primitive conditions of birth and death.

For those living in the modern way, any increase would be risky in the ultimate biological sense of the survival of the population. But I cannot believe that, when aware of these principles, society will continue indefinitely without the practice of a more salutary genetic selection, which would have to be consciously guided. And if this came to pass it should eventually be as possible for civilized populations to adjust to 20 r as for primitive ones to do so, although considerable inconvenience would be entailed thereby. Taking this into consideration, and also the fact that the effects of the 20 r would become evident only very slowly, I should be inclined not to set a lower limit than this at this moment. To attempt to do so might arouse so much opposition as to defeat the adoption of the policy. At the same time, it should be recognized that the lower the level which it is practicable to set, the better.

The above discussion concerns itself with the establishment of a per capita average ceiling, beyond which it is biologically dangerous for the species as a whole to go. This would however allow some individuals to receive a good deal more than the average, being limited only by opportunistic considerations and by the risk to themselves of somatic damage or sterility, provided others received enough less than the ceiling dose to keep the average below it. Moreover, it would not matter, so far as gene mutations are concerned, how the exposure of any given individual was distributed in time. We can be sure, in any case, that the genetic effects in the more immediate progeny of the more heavily exposed individuals would not be concentrated or conspicuous enough to be noticeable to the parents who had received the unusually high exposures. An exception may be made here only when a high dose, say over 20 r, was given within a period of a few weeks, and reproduction occurred within some weeks after it, for then chromosome abnormalities, leading to an inherited tendency to abortion, would be too apt to occur. Special rules should be drawn up to guard against the occurrence of such cases. Not only this condition, but also the establishment of an average per capita dose, would of course require an elaborate system of recording and accounting of exposures, but that is a requirement which will eventually have to be faced, and met, anyway.

It may be contended that in no modern population is the average per capita dose received by the gonads in the period of a reproductive gener-

ation, that is, from the time a person is conceived until he in turn conceives, approaching a figure as high as 20 r. I believe, however, that with the spread among the population in general of radiological examinations and treatments, and especially of fluoroscopy both in pregnancy and under other circumstances, plus the increasing use of radioactivity in many ways, such a situation is being approached.[1]

In this connection attention may be called to the recent studies by SONNENBLICK and his co-workers (1951, 1952), of the practices of physicians in the region of Newark, near New York. In this work it was found that not one among over 200 of the operators of roentgen ray machines for medical purposes knew what output their machines had. In consequence, some of them delivered about 40 times as high a dose per minute as others. Again, it should be recalled that a by no means negligible proportion of women have some inhibition of ovulation, and that the practice is being taken up on a considerable scale of overcoming this by doses of several hundred r delivered to the ovaries. The chief promoters of this practice, MAZER and IZRAEL (1941), HAMAN (1947), KAPLAN (1949), and RUBIN (1952), defend it on the ground that the resulting children have not shown a high frequency of conspicuous abnormalities. Disregarding genetic considerations, they conclude from these observations, which are essentially irrelevant (see MULLER, 1950, 1951, 1954), that the genetic effects are negligible. Similarly, the practice is said to be growing among American physicians of treating the testes of young men with some 500 r as a means of temporary birth control, to last several years, with the expectation of their later having children. At the same time, a *few* of those in prominent positions connected with the development of atomic energy, and more in radiological practice, are promoting a policy of minimizing and casting doubt on the genetic damage produced by radiation, even though at the atomic energy installations themselves great care is used to protect the personnel. As a result, some editors of important American magazines refuse to publish discussions of the subject.

In view of all this, and of the already quarter-century old antagonistic attitude toward the subject of the genetic damage of radiation on the part of a large proportion of medical and business men using radiation, it should be evident that the per capita exposure could easily rise beyond the 20 r per capita level which I have suggested as an ultimate ceiling, unless the genetic need of such a ceiling became generally accepted. The now promulgated permissible dose of 0.3 r per week, amount-

[1] Since the above was written confirmation of this opinion has been provided by the data published in the recently issued "Summary of the Reported Radiation Exposure in the U. S." (see list of references).

ing as it does to over 400 r in 26 years, that is, in a reproductive generation, is not unreasonable so long as it is confined to a relatively few people, but unless it is expressly qualified by a far lower general ceiling for the population as a whole, it is likely to be misunderstood and thus to encourage those who are leading us towards a dangerous per capita level.

It has been argued that the problem of preserving or improving the genetic quality of the population is a more general one than that of protecting the genetic material from radiation, and that other circumstances, such as the uncontrolled reproduction of those who are genetically more afflicted, and exposure to mutagenic chemicals, may be doing much more harm than radiation. All this is perfectly true. But when it is asserted, further, that this gives us a valid reason, in the meantime, to disregard the genetic effects of radiation, the argument becomes a specious one and in my opinion vicious. It is like saying that we should not have safety regulations for the driving of automobiles because we have not solved the problems of cancer or of war. It happens that our own problem is that of radiation, that the genetic damage of radiation is real, and that it will be exerted no matter whether or not the other dangers are overcome. Its effects are in any case added to theirs, and are not diminished, no matter how great those other effects may be. But perhaps if we can be rational in respect to radiation, this policy will have a salutary influence on men's attitudes towards the other matters also. To say that we should save money by being irrational or unethical in this field in order to provide funds for doing research on the other problems is in my opinion an inexcusable evasion of the issue confronting ourselves, for we know that the funds are not capable of being diverted in this way.

The establishment of a per capita average ceiling of 20 r, or 10 r, or whatever may be decided upon, is by no means the final desideratum in protection against the genetic damage of radiation, but is only a kind of stop-gap in the last line of defense. All radiation received by the gonads entails some risk of damage to later generations. And even if many persons receive so little radiation as to keep the average down to 20 r those who do receive substantial amounts will nevertheless pass along an increased genetic load which will in proportionate measure afflict posterity, regardless of the impossibility of recognizing and pinpointing just which effects are derived from the radiation. It should therefore be the aim of users of radiation to keep the exposure of the gonads to the lowest practicable level in all cases, both for patients and for those exposed to radiation in their work or as bystanders. And each kind of case must therefore be decided, to some extent at least, on its own merits.

The principles according to which it should be decided, in any given case, whether an exposure should be made, and how much it may be, are

from a theoretical standpoint clear, and have been stated in the beginning of our discussion. They involve the balancing of all the benefit, or probability of benefit, against all the damage, or probability of damage. But the actual decision involves much uncertainty on both sides of the scales, as is so often the case in medical decisions, and much intuitive judgment must unavoidably be used. Nevertheless there are certain concrete facts and figures which should enter into the reckoning.

So far as the genetic damage to posterity is concerned, we must for the present act on the assumption that the effects in man are quantitatively like those demonstrated by RUSSELL in mice. From these we estimate that the single or fractionated exposure to 80 r of the gonads of one individual who will thereafter have two surviving children will on the average result in at least one fifth of a genetic death on the part of some descendant. This means that among the descendants of five persons so exposed there will on the average be at least one such death. To go along with this, there will be a correspondingly increased genetic load, distributed among numerous descendants each having a small fraction of it, and if this load were gathered together into one descendant of each of the five exposed persons it would decrease his genetic fitness by about 20 % below that of the average individual. It can be seen that, in many cases, this disability would be greater than the amount of benefit conferred on the individual himself by the exposure, when the whole course of his own life is taken into consideration. And even if it were just equal, it can be seen that the procedure would be like that of restoring the lost hand of one person by transplanting onto him, were it possible, the normal hand of some one else, in a case where the second person was far away in space or time and would never be able to find out how he lost it, and where the excuse was made that *possibly* the other person was living under such a high civilization that a reasonably good artificial hand might be made for him, or even a new one grown.

In most cases however the question is not whether a given exposure or series of exposures is to be made at all, but whether it is worth while to modify it, as for instance by special shielding, regulation of field and direction, scatter, voltage and accompanying conditions, so that a smaller effective dose will be delivered to the gonads. A recent development along these lines is said to be the photoelectric amplification of the image so as to make a smaller dose possible in diagnosis. In some cases, on the other hand, quite other means than radiation can be found for achieving the given objective. Whenever there are any such alternatives, whether involving modification of the irradiation or substitution of another method, the principle for deciding whether to employ a given irradiation is to estimate, on the one hand, the probability of benefit by each method, and on the other hand the cost, not only in terms of inconvenience, un-

certainty and expense to the operator, but also in terms of damage to the patient himself and — more especially, for irradiation involving pelvic regions or the whole body — to his descendants. And whereas the benefit of a proposed type of exposure may often be reckoned to be greater than its cost, even when the cost to the descendants is included, it may nevertheless, in many cases, turn out that the balance of benefit minus cost is not as great for the proposed exposure as for one of the alternative procedures. The method showing the highest balance would then be the preferable one.

It is naturally impracticable to weigh all these uncertain factors in detail for each case separately. But most cases fall into one of a relatively small number of categories, and for each of these categories thinking should be carried out along these lines. Moreover, in view of the fact that there is no threshold for mutagenesis, so that even the smallest dose involves some genetic danger, and in view of the recent finding that this danger in mammals is probably greater than had been supposed even by those who insisted on its reality, it is not advisable to rest content with choosing between different alternative procedures which today present themselves. Ways can be found, if the will is there, of affording greater germinal protection than is now feasible; methods and apparatus can be worked out which when used on a wide scale will give better protection at much less cost and inconvenience than would at present be possible; and, for some types of cases, such as for example the overcoming of the block to ovulation, improved methods not involving radiation at all can be devised. Thus radiation will gradually be bereft of its damage and left to work little else than benefit. But the process of bringing this important reform about will be greatly accelerated if only it is clearly recognized by medical men and all others dealing with ionizing radiation that the genetic effect is a real one, that it lacks a threshold, and that the amount of genetic damage which would be caused by extending the procedures now so prevalent is a very substantial amount, for which our descendants would not thank us.

Conclusion

Each inherited mutation, no matter how minute, is ultimately a cause, usually in heterozygous condition, of the 'genetic death' of one descendant, on the average. As the number of descendants to which it becomes transmitted is inversely proportional to the damage it does to each individual, the total loss of fitness caused in the population is comparable for mutations having small and large effects. But the total frequency of genetic deaths may be only a half to a third as high as the frequency of mutations because of some mutations acting synergistically

in causing elimination. All individuals contain many mutant genes, whose cumulative action results in a 'load', or reduction of his fitness, which may be expressed as being the same in amount as his chance of genetic death.

Modern studies indicate that something of the order of 1 in 50,000 (0.00002) of human gametes receive a newly arisen spontaneous mutation at a given locus, or (if there are 15,000 loci) 0.3 at any locus, and therefore 0.6 of zygotes. Assuming, because of synergism, only a third as many deaths, it follows that 0.2, or 1 in 5 persons, would regularly meet genetic death under equilibrium conditions. There is not an equilibrium at present but one must eventually become established in relation to conditions of selection.

How now does the radiation effect compare with this spontaneous mutation rate? RUSSELL's data show that in mice a dose of 80 r induces 1 mutation in a given gene in 50,000 gametes. This dose then, if equally effective in humans, would probably give a mutation rate about equal to the spontaneous rate. Thus 80 r, if continued indefinitely, would result in a doubling of the total mutation rate, causing 2 in 5 persons to meet genetic death, and result in a 40 % (instead of the usual 20 %) reduction in the fitness of the average individual below the theoretical homozygous normal.

Under modern civilized conditions human beings do not have a high enough rate of reproduction, or one which is genetically differential enough, to compensate for a doubled amount of genetic death. The result, if such a situation persisted indefinitely, would be continued genetic deterioration and/or a continued decline in numbers, until extinction. According to the same principles of calculation, exposure of the gonads of just one reproductive individual in one generation to some 80 r would on the average give the equivalent of one fifth of one genetic death of a descendant, and a total load, distributed amongst all his descendants, equivalent to a 20 % loss of fitness on the part of one individual. This would usually be more than the increased benefit of the 80 r (as compared with alternative methods of treatment) for the exposed individual himself.

It can be calculated that a given exposure of the whole body or of the pelvic region causes much greater total harm to later generations than to the exposed individual himself. Therefore the genetic damage from such exposures should furnish the chief criterion for limiting their dose. According to present knowledge, it seems advisable, as a *provisional measure*, to limit the *average* per capita (including prenatal) dose received before reproduction to a quarter of that probably causing a mutation rate equal to the spontaneous one, *i. e.* to about 20 r. This amount would be received in 16 months if delivered at 0.3 r per week. However, some

individuals might be allowed to receive more if others received correspondingly less. This would, for the present, allow the permissible dose to remain at 0.3 r per week for decades for the relatively few persons occupationally exposed to radiation.

Yet, even within these limits, an individual exposure would be justified only if the reckoned greater harm to posterity was less than the greater benefit to be derived from that exposure as compared with some alternative procedure. It should be recognized that since all exposures of the gonads of reproductive individuals entail some risk of genetic damage they should always be reduced to a minimum by all feasible means. Moreover, ways should be sought of attaining equivalent results by other methods than irradiation.

SUMMARY

Investigations by RUSSELL in mice show that a dose of 80 r induces 1 mutation in a given gene in 50,000 gametes. If the same mutation rate applies to humans, it would probably result in a doubling of the spontaneous production of mutations and, if continued indefinitely, it would cause "genetic death" of 2 persons in 5. It would also result in a 40 % (instead of the usual 20 %) reduction in the fitness of the average individual. If such a situation persisted indefinitely, the result would be a continued genetic deterioration and/or a continued decline in numbers.

As a *provisional measure* it seems advisable to limit the *average* per capita dose received before reproduction to about 20 r, that is to a dose which causes 25 % of the spontaneous mutation rate.

ZUSAMMENFASSUNG

Untersuchungen RUSSELLS an Mäusen zeigen, dass eine Dosis von 80 r eine Mutation in einem bestimmten Gen bei 50,000 Gameten erzeugt. Falls die gleiche Mutationsfrequenz für den Menschen angewendet werden kann, würde diese wahrscheinlich in einer Verdoppelung der spontanen Mutationsproduktion resultieren und, falls dieses unbegrenzt weiter geschieht, würde sie den »genetischen Tod« von 2 unter 5 Personen erzeugen. Das Ergebnis würde also eine 40 %ige (an Stelle der üblichen 20 %igen) Herabsetzung der Lebensstärke des Durchschnittsindividuums sein. Falls eine derartige Situation dauernd bestehen bleibt, würde das Ergebnis eine fortschreitende genetische Verschlechterung und/oder ein fortgesetzter zahlenmässiger Rückgang sein.

Als *provisorische Massnahme* scheint es ratsam, die vor der Fortpflanzung erhaltene *durchschnittliche* Dosis per capita auf etwa 20 r zu begrenzen; diese Dosis erzeugt etwa 25 % der spontanen Mutationsfrequenz.

RÉSUMÉ

Les recherches de RUSSELL sur la souris montrent qu'une dose de 80 r provoque une mutation d'un gène donné sur 50,000 gamètes. Si le même taux de mutation s'appliquait aux humains, il doublerait probablement le nombre des mutations spontanées, et, continué indéfiniment, causerait la «mort génétique» de 2 personnes sur 5. Il causerait aussi

une réduction de 40 % (au lieu des 20 % habituels) de la résistibilité de l'individu moyen. Si une telle situation persistait indéfiniment, le résultat serait une détérioration génétique continue et/ou une baisse continue du nombre.

A titre de *mesure provisoire*, il semble judicieux de limiter la dose *moyenne* par individu reçue avant la reproduction à environ 20 r, c'est-à-dire à une dose qui cause 25 % du taux de mutation spontanée.

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FROM THE ATOMIC ENERGY RESEARCH ESTABLISHMENT, HARWELL, ENGLAND

THE WHITE CELL COUNT IN RELATION TO OCCUPATIONAL RADIATION DOSAGE

by

E. K. Williams

There is general agreement that the blood count and, in particular, the white cell count, is a useful measurable index of the effect of doses of radiation to the whole body.

It is customary to rely on leucocyte counts to give warning of over-exposure in those who are liable to occupational radiation dosage, and hence to assess the adequacy or otherwise of protective measures. The British Roentgen-Ray and Radium Protection Committee, 1948, recommend "If at any time there is found to be a decided and progressive drop in either the total leucocyte or the total lymphocyte count, the worker should cease work and be placed under treatment for an adequate period. On resumption of work every care should be taken and the circumstances of the work reviewed so as to prevent a recurrence."

The International Commission on Radiological Protection, whilst proposing a value for the maximum permissible exposure to the whole body, also strongly recommends that every effort should be made to reduce exposures to all types of ionizing radiations to the lowest possible level. The result of the application of this recommendation at A. E. R. E. is that the great majority of exposures are far below the permissible maximum. With reduction of the exposure level, it is reasonable to assume that less change in the white cell count will be seen until a level is reached at which no change is detectable.

It is necessary, therefore, to review the blood counting procedure and results. Work carried out at A. E. R. E. has sought to evaluate the technical variation of the white cell count (CHAMBERLAIN and TURNER, 1952) and the physiological variation of the white cell content of an individual's blood (CHAMBERLAIN, TURNER and WILLIAMS, 1952).

Technical Variation

The results show that the total coefficient of variation of an average leucocyte count of 7,000 leucocytes/mm³ due to instrumental variation and field error is approximately 8 %; for a leucopenia of 3,000 leucocytes/mm³ it is approximately 10 %. The evaluation of different types of cell, such as neutrophils and lymphocytes, is carried out by means of a differential count, and even when 200 cells are counted it is found that the contribution of the error of this differential count to the error of the neutrophil or lymphocyte count is considerable. For the average case, the coefficient of variation of the neutrophil count is 10 %, for the lymphocyte count 12 %, and for a less common cell such as the monocyte 20 %.

Physiological Variation

Large as this technical error appears, it is not the critical factor, as physiological variations in the white cell content of the blood are even greater. The literature on these physiological variations is considerable and in a review published 17 years ago GARREY and BRYAN (1935) list nearly 300 references. The causes of the variations are multiple, unrelated and imperfectly understood. Each may make a considerable contribution to the total effect.

The following facts of a qualitative nature are established:

(1) There is a diurnal variation in the total white cell count of normal subjects with a peak in the afternoon (SHAW 1927, SIMPSON 1933, JACOBSON and MARKS 1947, MARKS 1952).

(2) Exercise, emotional states and injection of adrenalin produce transitory leucocytosis in the peripheral blood (JACOBSON and MARKS 1947, MARKS 1952, ROBERTSON-SMITH and HAYHOE 1952).

Diurnal Variation

Using venous blood samples, MARKS (1952) shows in an analysis of 500 normal counts a rise for leucocytes, neutrophils and lymphocytes during the day to a maximum in the late afternoon, the differences being statistically significant.

SHAW's results, as regards the afternoon maximum, are confirmed by an analysis of 1,773 A. E. R. E. pre-employment counts according to the time of day at which the count was done. An evaluation of the coefficients of variation during the day in unexposed A. E. R. E. laboratory technicians gives 11 % leucocytes, 15 % and 16 % for neutrophils and lymphocytes respectively. In a recent extension of this work, which will be reported in full elsewhere, series of counts have been done at 5

minute intervals during the day. It appears that there is a short-term physiological variation which occurs in the leucocyte content of the blood over a period of a few minutes. SIMPSON (1933) noted this in a number of 5 minute counts on a single subject (himself). The mean coefficient of variation deduced from his series is 14.8 %; in our experiment it is 10.6 %, to which the technical variation contributes 7 % and the physiological variance 8 % (the combined coefficient is the square root of the sum of squares of the components). Thus the diurnal variation is one aspect of random short-term variations in the blood leucocyte content.

The day to day variation is greater. The coefficient of variation between successive counts on individuals derived from various blood count studies in the literature and from our own results are given.

Day to Day Variation of Leucocyte Counts

	GOODFELLOW 1936	BIGGS 1952	NICKSON 1952	A.E.R.E. 1952
Average coefficient of variation..	15 %	16 %	14 %	16 %

As the period between successive counts increases, the random variation between them increases. Counts made on alternate days for 30 days on unexposed nurses (WHITE PHILLIPS, 1933) have a coefficient of variation of 17 %, at monthly intervals on radium workers (GOODFELLOW 1936) 19 %, and at 6-monthly intervals on unexposed subjects (CHAMBERLAIN, TURNER and WILLIAMS 1952) 21 %.

Other Physiological Factors

There is evidence at A. E. R. E. that the initial counts on applicants for employment are higher than subsequent counts, whether there is exposure to radiation or not. The suggestion is made (DICKIE and HEMPELMANN, 1947) that some emotional stress accompanies the experience of initial blood sampling and produces in turn a slightly abnormal count for the individual. It is known that this results in temporary leucocytosis (GARREY and BRYAN, 1935). It is also known that emotional states are accompanied by a release of adrenalin. The effect of 1 ml of 1/1,000 adrenalin given subcutaneously is an increase in the total leucocytes, granulocytes and lymphocytes in capillary and venous blood samples to approximately twice the base-line figure in from 15 to 20 minutes (ROBERTSON-SMITH and HAYHOE, 1952). The lymphocytes return to normal within an hour, but the rise in granulocytes is much longer sustained.

The effect of the moderate amount of exercise entailed in cycling one mile at a reasonable speed is shown to cause a significant increase in the leucocyte count (MARKS, 1952).

Effect of standard exercise on the leucocyte count
50 tests (venous blood samples)

	Mean	Standard deviation
Before exercise	6,266	1,562
After exercise	8,781	2,331

An attempt, however, to reduce variations due to these causes or the technical variation below the level at which its contribution to the total time-based variation becomes small is pointless.

Variation in White Cell Count in Relation to Radiation Dosage

1) In relation to acute exposure.

In a comprehensive study of nine cases accidentally exposed to massive radiation dosage (HEMPELMANN, LISCO and HOFFMAN, 1952) an immediate and sustained drop in lymphocytes was noted in most of the exposed persons. All cases showed an initial leucocytosis due to an increase in the number of mature neutrophils. None of the patients showed clearcut neutropenia except for the most heavily exposed: the means all fell within the probable 95 % normal range (CHAMBERLAIN, TURNER and WILLIAMS, 1952).

The depression of the lymphocyte count below the pre-exposure level was persistent: in two cases it was below this in the 2nd year, though still within normal limits. Both men were fit and well. The doses were equivalent to 390 r of 80 kv roentgen-rays and 26 r of gamma rays in one case and 186 r of soft roentgen-rays and 10.7 r of gamma rays in the other.

These results confirm the findings on animals during and since the war that the lymphocytes are the most sensitive cells. Attempts at quantitative expression of the fall have been made by GOODFELLOW (1936), JACOBSON et al. (1947) and PRICE (1948). With GOODFELLOW's results there is some correlation of percentage fall with dosage — at 2,000 mgm hours the percentage daily fall is of the order of 5 % and at 15,000 mgm hours 10 to 15 %. Thus, with an integral dose of about 100 r whole body gamma radiation, GOODFELLOW found a 20 to 40 % drop at the end of seven days after this dose. PRICE (1948 and 1951) shows that the total lymphocyte count is related to the total dose of radiation given and there is a slower rate of fall in the higher dose ranges. From his mathematical function relating the integral dose of radiation to the percent survival of lymphocytes in the human body, it may be deduced that a dose of 5 megagram r or about 100 r total body gamma radiation is required to produce a 35 % fall. JACOBSON and others, using rabbits,

find a transient minimal fall after 25 r, with prompt recovery to normal, and a fall of 25 % in the 25—50 r range.

2) In relation to chronic low exposure.

The reported effects of small daily radiation doses on the peripheral blood have been summarized recently (STONE 1952, CHAMBERLAIN, TURNER and WILLIAMS, 1952). In animals, changes in total leucocyte and absolute lymphocyte levels do not appear at daily doses within the tolerance range. The lowest value given is that of INGRAM and MASON (1950), who record in dogs given 0.5 r daily, an apparent depression of lymphocytes after six months, statistically significant after two years. The same authors, however, find that 10 r per day is the smallest daily dose producing significant depression of the lymphocyte count in rabbits.

Pre-employment and subsequent white cell counts at A. E. R. E., analysed by radiation group, give some slim statistical evidence of an effect of radiation on the mean lymphocyte count of groups of workers regularly exposed at an average dose per year of less than 0.5 r. KNOWLTON (1949) analysed the counts of workers at Los Alamos and the results offer some evidence of an effect on all counts at approximately 0.2 r/week.

There is general agreement that an early change in the blood due to overexposure is a leucopenia. In radium workers, GOODFELLOW (1935) considers that an absolute and progressive leucopenia due to a reduction in neutrophils is the only sign of overexposure common to all workers, and notes that in radium therapy (1936) lymphocytes are the blood cells most affected. Leucopenia and a reduction in the percentage of granulocytes with a relative lymphocytosis is noted by NORDENSON (1946). JACOBSON and MARKS (1947) consider "lymphocyte reduction in the peripheral blood is the most sensitive indication of acute or sub-acute exposure to externally originating ionizing radiation or internally deposited radioisotopes" as a result of extensive animal experiments.

The Normal Count

In a series of 3,098 pre-employment blood counts at A. E. R. E., six per cent of leucocyte counts and 14 per cent of lymphocyte counts fall below the levels 5,000 and 1,500 respectively given in the 1943 edition of the Recommendations of the British Roentgen-ray and Radium Protection Committee as the lower limits of normal.

The question has been raised as to whether apparently normal healthy individuals with a persistently low leucocyte count run a greater risk from exposure to radiation than those with counts in the so-called

Lower limits of Normal (95 % range)

	A. E. R. E., England (3,098 observations)	Blackburn, Australia (642 observations)
Leucocytes	4,500	3,082
Neutrophils	2,250	856
Lymphocytes	1,100	882

"normal" ranges. It is tacitly assumed that any further depression of granulocytes would reduce resistance to infection. An investigation has been made at A. E. R. E. into the relative sickness rates of individuals with persistent leucopenia and those with normal counts. For the purpose of this investigation the criteria adopted were leucocytes $< 5{,}000$, polymorphs $< 3{,}000$, lymphocytes $< 1{,}500$ per c/mm. On the evidence obtained at A. E. R. E., there is no support for the supposition that individuals with a white cell count persistently below these limits are either more susceptible to illness or react less favourably to infection than those with counts in the so-called normal range (TURNER, 1952).

From a study of the cases described in "The Acute Radiation Syndrome" (HEMPELMANN, LISCO and HOFFMAN, 1952) it is difficult to assess the importance to be attached to the persistent reduction in lymphocytes following the accidental high exposure.

Cytologic Changes in Leucocytes

In their study it is of considerable interest to note the occurrence of an increased number of refractive, neutral-redstaining granules in the lymphocytes of all but two of the cases. Moreover, this increase persisted for over one year in one case and for more than two years in another. This change is recorded in individuals occupationally exposed to radiation (DICKIE and HEMPELMANN, 1947) and in patients with beta ray burns (KNOWLTON et al., 1949). This effect is not specific for radiation since it is also observed in the lymphocytes of persons exposed to certain toxic chemicals, but it may prove a useful guide to exposure. Degenerative changes in neutrophils and lymphocytes were seen in the severely injured patients. The occurrence of pathologic lymphocytes in the blood pictures of workers with roentgen rays is given by HELDE (1946) as an indication of bad conditions at roentgen establishments. GOODFELLOW (1935) notes "the appearance of Türk cells or embryonic white cells in the blood coincided with such occasions of excessive exposure as to leave no doubt that irradiation was responsible".

In massive dose radium therapy he notes that immature forms are common, and cites lymphocytes exhibiting evidence of amitotic division and atypical monocytes (GOODFELLOW, 1936). BROWNING (1949) notes

abnormal monocytes in the blood of luminisers and the disappearance of these cells after the cessation of exposure. The occurrence of lymphocytes with bilobed nuclei in cyclotron personnel is reported by INGRAM and BARNES (1949), and the relationship between the increased incidence of these cells and exposure to very small amounts of radiation from the cyclotron is confirmed by experiments on dogs (INGRAM and BARNES, 1950).

The incidence was high in the cyclotron group during the early months of operation, fell and remained low after completion of a protective dyke. "The findings are directed particularly to the attention of persons concerned with the prevention of radiation injuries because the increased incidence of binucleate lymphocytes appears to be related to exposure to exceptionally small amounts of radiation" (INGRAM et al., 1952). It is suggested that "examination for the presence of these cells serves as a practicable and sensitive adjunct to physical monitoring".

Recommendations and Conclusions

In estimating quantitative changes in the white cell content of the blood, the technical error and coefficient of variation due to physiological causes should be taken into account. Otherwise conclusions as to the contribution of radiation effect may be invalidated and the counting procedure fall into disrepute.

STONE (1952) asserts that thousands of blood counts are being done in cases where good protection is afforded by monitoring with radiation instruments and considers "this is a terrific waste of money, time, manpower and effort" and "mass counting should be discontinued". Such a statement coming from one with truly vast experience cannot be dismissed lightly, nor can his further proposal for work on an experimental basis. The need is felt for reliable data linking blood counts of selected groups with accurate measurements of radiation exposure, and for data on a number of specific controls. Consideration should therefore be given to selective processes whereby greater effort is expended when the risk is higher or when positive results may be obtained, with concomitant relaxation on "potentially exposed" personnel who are also monitored. Some of the effort gained in this way might well be employed on further study of cytologic changes which may prove a more sensitive index of radiation effect than gross numerical variation and aid us further in our task of protection of workers exposed to radiation.

Acknowledgment

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SUMMARY

The extent of the technical variation in the white cell count is considered. Physiological variations in the leucocyte content of the blood are considered and, in particular, the extent of variation to be expected from random time-based fluctuations.

Results in the literature are examined as to the effect and extent of radiation exposure on the white cell count. Cytologic changes suggested as an index of radiation effect are described.

ZUSAMMENFASSUNG

Das Ausmass der technischen Variation im weissen Blutbilde wird einer Beurteilung unterzogen. Physiologische Schwankungen des Leukozytenhaltes des Blutes und besonders das Ausmass der durch zufällige zeitgebundene Fluktuationen zu erwartenden Variationen werden betrachtet.

Veröffentlichte Ergebnisse werden hinsichtlich der Wirkung und des Ausmasses der Bestrahlung auf die Anzahl der Leukozyten untersucht. Zytologische Veränderungen, die als ein Index der Strahlenwirkung angesehen werden, werden beschrieben.

RÉSUMÉ

L'auteur étudie l'étendue des variations dues à la technique dans la numération des leucocytes. Il étudie les variations physiologiques du nombre des leucocytes dans le sang et, en particulier, l'étendue des variations dues aux fluctuations accidentelles en fonction du temps.

Il examine les résultats parus dans la littérature sur la nature et l'étendue des effets de l'exposition aux radiations sur la numération leucocytaire. Il décrit des modifications cytologiques proposées comme indice de l'effet des radiations.

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MAXIMUM PERMISSIBLE CONCENTRATION OF RADIOISOTOPES IN FOOD, WATER AND AIR AND MAXIMUM PERMISSIBLE EQUILIBRIUM AMOUNTS IN THE BODY

by

Karl Z. Morgan

Introduction

The Subcommittee on Internal Dose of the National (U. S.) Committee on Radiation Protection has prepared a handbook (1) dealing with the problems of internal dose from ionizing radiations. This handbook gives tables, listing values for the maximum permissible body burden and the maximum permissible concentration in air and in water of seventy radioisotopes. These maximum permissible values are considered to be applicable both to occupational exposure and exposure to large populations. Although there may be sound argument from the standpoint of genetic effects for reducing the permissible exposure to large populations, it is not considered desirable to establish two sets of standards — one for the worker and another for the population at large. The attitude of the committee has been that all unnecessary accumulation of radioisotopes in the body should be avoided and that the radiation hazards in industry should be so low as not to constitute an appreciable added risk or require special hazards pay. This does not mean that all radioisotopes should be kept out of the body because such a restriction would be extremely costly and would unnecessarily hamper development in the field of atomic energy. Rather, it means the radiation hazards both from internal and external sources of ionizing radiation should be kept so low that they can be considered to result in estimated damage equal to or less than that resulting from ordinary and willingly

accepted overindulgence — such as overeating, loss of sleep, improper clothing, etc. In other words, the maximum permissible radiation exposure would not result in any readily detectable biological damage.

Internal exposure to radiation workers is of greater concern than external radiation exposure because: 1) it represents a continuous exposure 24 hours a day that may continue for many days, even years; 2) the radioisotopes are in intimate contact with critical body tissues so that alpha and beta radiation are also very effective in producing damage; and 3) it is difficult to make quantitative estimates of the body burden and even more difficult to accelerate the removal of the radioactive material once it becomes fixed in the body.

Many uncertainties exist in the biological data that must be used in establishing satisfactory maximum permissible body burdens for these radioisotopes or maximum permissible concentrations in food, water and air. However, thousands of people already are working with these radioactive materials and many others are subject to possible exposure from radioactive contamination in the air and in the food and water so that it is expedient to select some maximum permissible values — however uncertain they may be — that represent the best estimates at the present time. It is the policy of the National (U. S.) Committee on Radiation Protection to avoid putting such regulations into law in order to maintain their flexibility and to simplify the procedures for the frequent revision necessary as more information becomes available. Because of the uncertainties in these values (given in Table I) the Committee is suggesting that a factor of safety (that may be as large as 10) be used in the design and operation of permanent installations where large quantities of radioactive materials are involved. This suggestion is made in order to prevent the build-up and accumulation of large quantities of radioactive contamination in the environment, so that should the values of the maximum permissible concentration be lowered in the future new standards could be met without delay and prohibitive cost.

The report of the Committee (Table I) lists values of maximum permissible body burden and maximum permissible concentrations in air and water for the equilibrium condition — that is, the rate of elimination of the radioisotope is equal to the rate of intake. In general one is concerned with all methods of intake — from food, water, air, through wounds, abrasions, and the pores of the skin — and with periods of exposure that may be instantaneous or extend throughout the lifetime of the individual. The maximum permissible concentration in food can be taken perhaps the same as in water. The maximum permissible amount of radioactive contaminant in a wound depends upon the efficiency with which the wound can be cleansed (or in some cases excised) and upon the rate of translocation from the wound to other body tissue. In most

cases it is probably safe to assume that most of the radioactive material will remain in the body — near the site of the wound or be translocated in the body — unless it is flushed out immediately after the accident or the contaminated tissue is excised. The penetration through pores of the skin depends upon the particular compound involved, and thus an effort should be made to avoid all contact with radioactive compounds that are highly permeable to the skin. Although Table I as given in the Handbook on Internal Dose lists only the values of maximum permissible concentration in air and water for the equilibrium condition, the complete report gives the initial retention and elimination factors and the necessary equations so that one can calculate the maximum permissible concentrations of the various radioisotopes for any period of exposure.

Radioisotopes that have become fixed in the body are eliminated primarily by way of the urine and feces or in a few cases by exhalation. On the AEC projects there have been developed methods for the analysis of body excretions for a number of radioactive elements to give a quantitative measurement of the daily elimination. This information is the basis for an estimate of the body burden of a radioisotope.

Criteria for Choosing the Maximum Permissible Amount of a Radioisotope in the Body

There are a number of methods that can be used for arriving at the maximum permissible values of the various radioisotopes. The only accurate method is by human experiment. This method of direct approach cannot be considered for ethical reasons except in a few special cases such as when an accident occurs in which one inhales or ingests a considerable amount of a radioisotope and the excretions can be analyzed to determine the biological half-life and the case where very small and completely harmless doses of certain radioisotopes can be administered to terminal patients and autopsy studies made to determine the distribution in the various body organs. Dr. Isabel H. Tipton (working on a joint project with the Health Physics Division of the Oak Ridge National Laboratory and the University of Tennessee) is making a direct approach to the problem of the distribution of elements by collecting samples of human tissue from body organs of persons that lived in a great variety of places in the United States and examining this tissue spectroscopically to get the stable element distribution. Ordinarily, under equilibrium conditions one would expect a radioactive isotope to follow the same body distribution as the stable isotope. Likewise, this spectroscopic method can be used to determine the element distribution in the average diet and from the two spectroscopic studies obtain the useful relationship

$$T_b = \frac{0.693\ M}{I\ f}$$

in which M = gm of an element in a particular body organ, I = gm per day of the element taken into the body, f = fraction of that element going to the body organ and T_b = biological half-life of the element in the organ.

Usually the human data needed for these calculations are not available and thus it becomes necessary to rely on data from animal experiments. While a large mass of animal data is available for some of the compounds of a few of the radioactive elements, in most cases there are no data available and one is compelled to make assumptions that are little better than guesses.

In the few cases where the chemistry of the radioisotope is similar to that of radium it is possible to utilize the 25 years of experience in observing and studying the radium distribution in the body and the effects produced. In addition, it is possible to compare the damage from internal doses of ionizing radiation with that from roentgen ray exposures. After 50 years of experience with roentgen rays, it is generally believed that no serious consequences will result if the dose rate to the total body is kept below 0.3 roentgen per week. Therefore, it is reasoned that the maximum permissible body burden of a radioisotope is one that does not irradiate any of the body organs at an average dose rate in excess of 0.3 roentgens (or rems) per week. It will be shown later (Table III) that this is not necessarily a safe assumption because the elements in question may follow a distribution within the organ that is not uniform and lead to local dose rates that are far in excess of the average.

In estimating the maximum permissible internal exposure the lower limit is determined usually by the level of natural or background radiation which probably averages about 0.4 mrep per day to certain of the critical organs. Since this background radiation varies considerably it would seem unreasonable to set the maximum permissible body burden of any of the radioactive isotopes, such as potassium, for example, at a value less than the normal amount of K^{40} in the body. Similarly one would not set the maximum permissible concentration in air and water of radioisotopes that behave chemically like radium at values less than the average concentration of radium found in nature.

Factors that Determine the Relative Radiation Hazards of Radioisotopes

Since most of these factors are apparent, it should be sufficient to list them with merely a word of explanation.

1. *Quantities Available.* Some radioisotopes are not available in sufficiently large quantities because of limited production facilities, short radioactive half-life or limited use to present a radiation hazard.

2. *Initial Body Retention.* The chemical and physical form of a radioisotope determines its initial retention in the body. The values given in Table I apply only to the more common chemical forms and in a few cases maximum permissible values are listed both for soluble and insoluble compounds. The initial retention in the gastro-intestinal tract ranges from 0.01 % for plutonium to 100 % for tritium. In the case of airborne particles the size, shape and electrical charge are important factors in determining the initial retention in the lungs.

3. *Fraction Going from Blood to Critical Organ.* Some elements are eliminated rapidly from the blood streams while large fractions of others are deposited in certain body organs.

4. *Radiosensitivity of Tissue.* Some tissues such as lymphatic tissue are more radiosensitive than others such as muscle tissue; thus, the same dose of ionizing radiation delivered to each produces the greater damage to the lymphatic tissue.

5. *Size of Critical Organ.* For a given amount of a radioisotope in an organ the average dose of ionizing radiation to the organ is inversely proportional to the mass of the organ.

6. *Essentialness of an Organ.* Two body organs may receive the same tissue damage, yet the damage to one organ may result in greater damage to the body as a whole because that organ is more vital to total body functioning. *The critical body organ* as used here is not necessarily that body organ with the greatest concentration of a radioisotope or even the one that receives the greatest damage, rather, it is the organ receiving the radioisotope that results in the greatest overall damage to the body.

7. *Biological Half-Life.* This is the time required for half of the radioisotope to be removed from the organ by normal biological processes.

8. *Radioactive Half-Life that is Approximately Equal to the Average Life Span of Man.* A radioisotope such as O^{19} with a radioactive half-life of 29 seconds presents no problem of internal dose. On the other hand a radioisotope such as U^{238} presents greater problems of chemical toxicity than of radiation damage because of its very low specific activity resulting from its long radioactive half-life of 4.5×10^9 years. One reason Sr^{90} presents one of the greatest internal dose hazards — in addition to being an isotope with many useful applications, a long biological half-life, a large initial retention factor and a large concentration in the critical tissue of bone — is that it has a half-life of 25 years which is a large fraction of the adult life of man. The biological half-life, T_b, and radioactive half-life, T_r, are treated together in the calculations as a single term called the *effective half-life*, T, which is given by the equation,

$$T = \frac{T_b \, T_r}{T_b + T_r} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (2)$$

9. *Effective Energy per Disintegration.* Pu^{239}, an alpha emitter with an energy of 5.16 Mev, presents a much greater radiation hazard than C^{14}, a beta emitter with a maximum energy of 0.15 Mev or an effective energy of about 0.05 Mev. The effective energy per disintegration of gamma radiation is less than the maximum energy because a large fraction escapes from the organ. The effective energies per disintegration in terms of the maximum energy is given approximately by the equations:

$$(E_{eff})_\alpha = (E_{max})_\alpha \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (3)$$

$$(E_{eff})_\beta = 0.33 \left(1 - \frac{Z^{1/2}}{43}\right)\left(1 + \frac{(E_{max})_\beta^{1/2}}{4}\right)(E_{max})_\beta \quad \ldots\ldots\ldots \quad (4)$$

$$(E_{eff})_\gamma = (1 - e^{-\sigma X})(E_{max})_\gamma \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (5)$$

in which Z = atomic number of the radioisotope, σ = total coefficient of absorption minus the Compton scattering coefficient in tissue in cm^{-1} and X = effective diameter of the body organ.

10. *Specific Ionization. The relative biological effectiveness (RBE)* of radiation for most gross biological damage increases with the specific ionization or ion pairs produced per cm path of the radiation in tissue and varies greatly with the type of biological damage under consideration. The most appropriate values of RBE for the various types of radiation are unknown so somewhat arbitrarily the RBE values for the various types of radiation have been chosen as follows:

1) for X, γ and β an RBE = 1, 2) for protons and fast neutrons an RBE = 10, and 3) for α radiation and heavy recoil ions an RBE = 20.[1]

Calculation of Maximum Permissible Internal Dose from Radioisotopes for the Equilibrium Condition

A few of the maximum permissible values given in Table I were selected on the basis of specific biological information but in most cases they were calculated applying limited biological data and making use of certain simplifying assumptions. It was assumed that a certain fraction of the inhaled or ingested radioisotope arrived at the critical organ where it was deposited uniformly and that it was eliminated exponentially with an effective half-life as given by Equation 2. For the values given in Table I no correction was made for the non-uniformity of deposition within the critical organ. This problem is discussed in the section dealing

[1] The HARRIMAN, N. Y. Conference on Radiation Protection meeting in April 1953 decided to reduce the RBE of α radiation from 20 to 10.

with "the Particle Problem". The permissible quantity of a radioisotope in a body organ was considered to be that amount that would result in an average dose rate of 0.3 rem per week. When more than one radioisotope is deposited in the body the maximum permissible body burden of the mixture is considered to be that amount that will result in a dose of 0.3 rem per week to any organ. There is indication of a synergistic effect in some cases when large doses of certain of the radioisotopes are administered simultaneously. However, there is no convincing evidence that the maximum permissible dose rate to tissue should be less than 0.3 rem per week because of the inter-dependence of body organs when radioisotopes are concerned in small quantities under equilibrium conditions and deposited in interdependent organs. For most of the radioisotopes equlibrium is reached after a few months exposure and in less than 20 years for all those listed in Table I with the exception of Pu^{239}, Sm^{151}, Ra^{226}, and Sr^{90}. The time of exposure used in the calculation of a maximum permissible value is not critical for most radioisotopes and if taken as 70 years for Pu^{239}, Sm^{151}, Ra^{226}, and Sr^{90}, they will reach 34 %, 36 %, 67 % and 100 % of equilibrium, respectively. In every case the values of maximum permissible concentration as given in Table I are such that the rate of exposure to the critical organ is 0.3 rem per week after a period of exposure equal to or less than 70 years. In most cases, however, this dose rate is reached and becomes constant after only a few months exposure as indicated in Diagram 1 which is a plot of the dose rate to the critical organ as a function of the period of exclusive use of water containing the indicated radioisotopes in a concentration as noted on each curve. In all of these calculations, the values used for the mass of body organs, breathing rate, rate of water intake and urine excretion rate are those for the Standard Man as agreed upon at the Chalk River Conference. (The Chalk River Conference was a meeting of the persons interested in radiation protection from the United States, Great Britain and Canada at Chalk River, Canada, September 29 and 30, 1949.)

The equation used for obtaining the maximum permissible body burden of a radioisotope is:

$$Q = \frac{2.6 \times 10^{-3}\ m\ W}{\Sigma (E_{eff})\ f_2} \qquad \ldots\ldots\ldots\ldots\ldots\ldots\ (6)$$

in which $Q = \mu c$ in total body, f_2 = fraction in critical organ, $\Sigma(E_{eff})$ = total effective energy in Mev, m = mass of critical organ in gm and W = 0.3/RBE rep per week. The rep corresponds to an energy absorption of 93 ergs/gm of tissue.

The maximum permissible concentration of a radioisotope in air is given by the equation,

$$(MPC)_a = \frac{3 \times 10^{-6}\ Q\ f_2}{T\ f_a\ (1 - e^{-0.693\ t\ T})} \qquad \ldots\ldots\ldots\ldots\ldots\ (7)$$

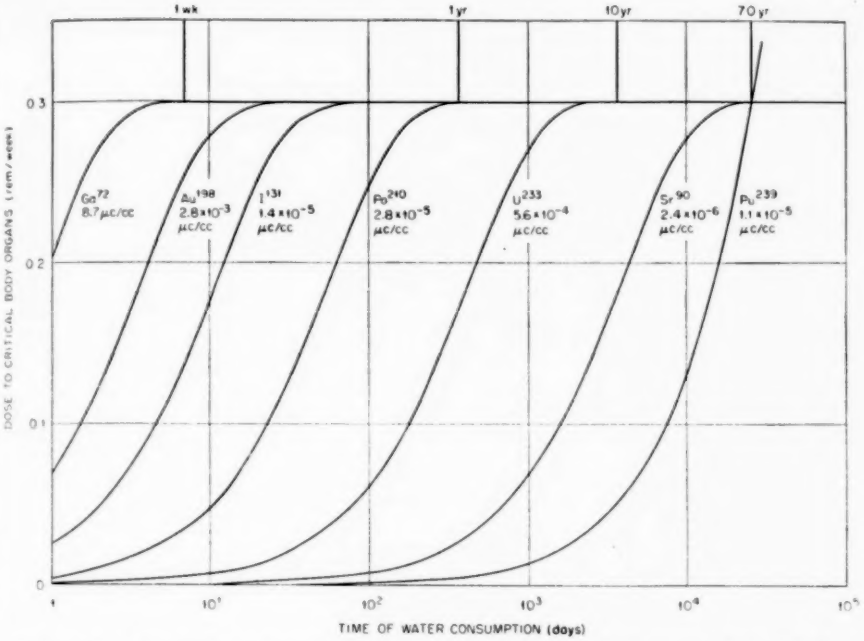

Diagram 1. Showing the increase in dose rate to the critical organ when drinking water is contaminated with radioisotopes at concentrations as indicated on each curve.

in which $(MPC)_a = \mu c$ per cc of air, T = effective half-life given in days, f_o = fraction of the inhaled radioisotope that is retained in the critical organ and t = time of exposure.

In the case of a noble gas the problem is one of submersion in a radioactive fluid and in this case the maximum permissible concentration of a radioisotope in air is given by the equation,

$$(MPC)_a^1 = \frac{0.8 \times 10^{-6}}{\Sigma (E_{eff})} \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (8)$$

in which $(MPC)_a^1 = \mu c$ of a noble gas per cc of air.

The maximum permissible concentration of a radioisotope in water is calculated using the equation,

$$(MPC)_w = \frac{3 \times 10^{-4}\, Q\, f_2}{T\, f_w\, (1 - e^{-0.693\, t\, T})} \quad \ldots\ldots\ldots\ldots\ldots \quad (9)$$

in which $(MPC)_w = \mu c$ per cc of water and f_w = fraction of that ingested radioisotope that is retained by the critical organ.

Table I

Maximum Permissible Amount of Radioisotope in Total Body and Maximum Permissible Concentration in Air and Water for Continuous Exposure.

Element	Organ	μc in Total Body	μc/cc Water	μc/cc Air
Unidentified β, γ	—	—	1×10^{-7}	1×10^{-9}
Unidentified α	—	—	1×10^{-7}	5×10^{-13}
U-nat (sol)	Kidney	0.02 *	7×10^{-5} *	1.7×10^{-11} *
U-nat (insol)	Lungs	0.009	—	1.7×10^{-11} *
U-233 (sol)	Bone	0.04	1.5×10^{-4}	1×10^{-10}
U-233 (insol)	Lungs	0.008 *	—	1.6×10^{-11} *
Ra-226 + 1/2 dr	Bone	0.1	4×10^{-4}	8×10^{-12}
Rn-222 + dr	Body	—	2×10^{-6}	1×10^{-8}
Pu-239 (sol)	Bone	0.04	1.5×10^{-6}	2×10^{-12}
Pu-239 (insol)	Lungs	0.008 *	—	2×10^{-12}
Po-210 (sol)	Spleen	0.02 *	3×10^{-5}	2×10^{-10} *
Po-210 (insol)	Lungs	7×10^{-3} *	—	7×10^{-11} *
C-14 (CO_2)	Fat	250	3×10^{-3}	1×10^{-6}
	Bone	1,500	4×10^{-3}	5×10^{-7}
H-3 (HTO or H^3_2O)	Total Body	10^4	0.2	2×10^{-5}
Ca-45	Bone	65 ∓	5×10^{-4} ∓	3×10^{-8} ∓
P-32	Bone	10	2×10^{-4}	1×10^{-7}
K-42	Muscle	20	1×10^{-2}	2×10^{-6}
S-35	Skin	100	5×10^{-3}	1×10^{-6}
Na-24	Total Body	15	8×10^{-3}	2×10^{-6}
Cl-36	Total Body	200	2×10^{-3}	4×10^{-7}
Fe-55	Blood	1×10^3	4×10^{-3}	6×10^{-7}
Fe-59	Blood	11	1×10^{-4}	1.5×10^{-8}
Mn-56	Kidneys	2	0.15	3×10^{-6}
	Liver	7.5	0.3	4×10^{-6}
Cu-64	Liver	1.5×10^2	8×10^{-2}	6×10^{-6}
I-131	Thyroid	0.3	3×10^{-5}	3×10^{-9}
Zn-65	Bone	430	6×10^{-2}	2×10^{-6}
Sr-89	Bone	2	7×10^{-5}	2×10^{-8}
Sr-90 + Y-90	Bone	1	8×10^{-7}	2×10^{-10}
A-41	Total Body	30	5×10^{-4}	5×10^{-7}
Xe-133	Total Body	300	4×10^{-3}	4×10^{-6}
Xe-135	Total Body	100	1×10^{-3}	2×10^{-6}
Co-60	Liver	3	2×10^{-2}	1×10^{-6}
Au-198	Kidneys	10	3×10^{-3}	1×10^{-7}
Au-199	Kidneys	28	7×10^{-3}	2.5×10^{-7}
Cr-51	Kidneys	390	0.5	8×10^{-6}
Ni-59	Liver	39	0.25	2×10^{-5}
Mo-99	Bone	50	14	2×10^{-3}
Th-234	Bone	120	3	6×10^{-7}
As-76	Kidneys	10	0.2	2×10^{-6}
Ga-72	Bone	8	9	3×10^{-6}
Ba-140 + La-140	Bone	5 ∓	2×10^{-3} ∓	6×10^{-8} ∓
F-18	Bone	24 ∓	0.9 ∓	1×10^{-4} ∓
	Teeth	0.07	2.5×10^{-3}	3×10^{-7}
Be-7	Bone	670	1	4×10^{-5}
Sc-46	Spleen	6	0.4	7×10^{-8}
V-48	Bone	20	0.5	1×10^{-6}
Ge-71	Kidneys	67	9	4×10^{-5}
Rb-86	Muscle	60	3×10^{-3}	4×10^{-7}
Y-91	Bone	15 ∓	0.2 ∓	4×10^{-8} ∓
Nb-95	Bone	90	4×10^{-3}	4×10^{-7}

Table I (Continued)

Element	Organ	μc in Total Body	μc/cc Water	μc/cc Air
Te-96	Kidneys	5	3×10^{-2}	3×10^{-6}
Ru-106 — Rh-106	Kidneys	4	0.1	3×10^{-8}
Rh-105	Kidneys	9	1.5×10^{-2}	8.5×10^{-7}
Pd-103	Kidneys	6	1×10^{-2}	7×10^{-7}
Cd-109 — Ag-109 m	Liver	40	7×10^{-2}	7×10^{-8}
Sn-113	Bone	80	0.2	6×10^{-7}
Te-127	Kidneys	4	3×10^{-2}	1×10^{-7}
Te-129	Kidneys	1.3	1×10^{-2}	4×10^{-8}
Cs-137 — Ba-137 m	Muscle	90	1.5×10^{-3}	2×10^{-7}
La-140	Bone	24 ∓	1 ∓	1×10^{-6} ∓
Re-183	Thyroid	35	8×10^{-2}	8×10^{-6}
	Skin	600	0.2	2×10^{-5}
Ir-190	Kidneys	21	1×10^{-2}	7×10^{-7}
Pb-203	Bone	57	0.1	6.5×10^{-6}
At-211	Thyroid	6×10^{-4}	2×10^{-6}	2×10^{-10}
Am-241	Bone	0.06	1×10^{-4}	3×10^{-11}
Cm-242	Bone	0.05	9×10^{-4}	2×10^{-10}
Ce-144 — Pr-144	Bone	5 ∓	4×10^{-2} ∓	7×10^{-9} ∓
Pr-143	Bone	29 ∓	4×10^{-1} ∓	7×10^{-7} ∓
Pm-147	Bone	120 ∓	1 ∓	2×10^{-7} ∓
Sm-151	Bone	420 ∓	0·2 ∓	1×10^{-8} ∓
Eu-154	Bone	22 ∓	3×10^{-2} ∓	6×10^{-9} ∓
Ho-166	Bone	17 ∓	23 ∓	3×10^{-6} ∓
Tm-170	Bone	19 ∓	2.5×10^{-1} ∓	5×10^{-8} ∓
Lu-177	Bone	78 ∓	24 ∓	5×10^{-6} ∓
Ag-111	Liver	36	4	3×10^{-5}
Ag-105	Liver	18	2	1×10^{-5}
W-181	Bone	32	1.6×10^{-1}	5×10^{-6}
Au-196	Liver	8	5×10^{-2}	2×10^{-7}
Tl-200	Muscle	37	2×10^{-2}	2×10^{-6}
Tl-201	Muscle	290	7×10^{-2}	7×10^{-6}
Tl-202	Muscle	220	2×10^{-2}	2×10^{-6}
Pt-191	Kidneys	2	6×10^{-3}	2×10^{-7}
Pt-193	Kidneys	3	5×10^{-3}	2×20^{-7}
Ir-192	Kidneys	3	9×10^{-4}	5×10^{-8}

Note: A number of changes were recommended in the values given in Table 1 as a result of the HARRIMAN, N. Y. Conference on Radiation Protection in April 1953. The most important change was to use the gastrointestinal tract as the critical organ in cases where it received the greatest dose. Other general changes in the above values were:

* double certain calculated valves for α-emitting radioisotopes because of the decrease in RBE from 20 to 10.

∓ reduce values for certain of the β-emitting radioisotopes that are bone seekers by a factor of 5 to account for non-uniform distribution in the bone.

Sometimes in practice it may not be practical to identify immediately the radioisotopes that are responsible for the contamination of the air and water. It is for this reason that values are given for "unidentified β and γ" and "unidentified α" emitting radioisotopes in Table I. These values are based on gross $\beta\gamma$ or gross α measurements which are much easier to make than radioisotope analysis and these values can be used safely for many months without the necessity of identifying the radioactive contamination.

Calculation of Maximum Permissible Concentration of Radioisotopes in Air and Water During Emergency Conditions

During emergency conditions following an accident or the use of atomic weapons it would not be realistic to use the values for maximum permissible concentration in air and water as given in Table I. There are several reasons why emergency values can be higher:

1) Radiation hazards should be permitted which are of the same order of magnitude as the other hazards incident to the emergency;

2) In case of atomic weapons the half-lives of the radioisotopes present would probably be very short; the half-life of the mixture of fission products being equal approximately to the time since the explosion;

3) The per cent of the more hazardous radioactive contaminants present immediately following an atomic explosion would be very low; and

4) The period of exposure in the emergency area usually would be short.

The National (U. S.) Committee on Radiation Protection has not selected any values of maximum permissible concentration for emergency situations but has indicated approval of the emergency external exposure dose of 25 rem to the total body as was approved by the Chalk River Conference. Several writers such as BALE (2) have suggested general values of maximum permissible concentration of mixed fission products in air and water following an atomic explosion but these values apply only to a particular period following the explosion. MORGAN and STRAUB (3) have developed equations that can be used to obtain the emergency maximum permissible concentrations in air and water that apply to various situations and for any time interval. The remainder of this discussion will deal briefly with some of their equations and tables of values.

Under emergency conditions the maximum permissible concentrations of a radioisotope in water and air are given by equations 10 and 11, respectively,

$$(\mathrm{MPC})_w^{\mathrm{emerg.}} = \frac{4 \times 10^{-6}\ \mathrm{r\ m\ X}}{(\mathrm{E_{eff}})\ \mathrm{f_w\ T\ T_r}} \quad \ldots\ldots\ldots\ldots (10)$$

$$(\mathrm{MPC})_a^{\mathrm{emerg.}} = \frac{4.4 \times 10^{-10}\ \mathrm{r\ m\ X}}{(\mathrm{E_{eff}})\ \mathrm{f_a\ T\ T_r}} \quad \ldots\ldots\ldots\ldots (11)$$

in which m, E_{eff}, f_w, f_a, T and T_r have the same definitions as in the previous equations, $(\mathrm{MPC})_w^{\mathrm{emerg.}} = \mu c/cc$ of water and $(\mathrm{MPC})_a^{\mathrm{emerg.}} = \mu c/cc$ of air present when the exposure began and which will deliver a dose r (r = reps) in a total time t_2. The time during which the radioisotope is

Table II

Tentative Maximum Permissible Concentration in Water for Emergency[1] Conditions.

Radioisotope	Time of Intake $t_1 = 1$ day		Time of Intake $t_1 = 30$ days	
	Critical Organ	μc-cc of Water	Critical Organ	μc/cc of Water
Co^{60}	G I Tract	2×10^{-1}	G I Tract	7×10^{-3}
$Sr^{90} + Y^{90}$	Bone	2×10^{-3}	Bone	5×10^{-5}
Pu^{239} (sol)	G I Tract	6×10^{-2}	G I Tract	2×10^{-3}
I^{131}	Thyroid	1×10^{-2}	Thyroid	8×10^{-4}
$Ce^{144} + Pr^{144}$	G I Tract	2×10^{-1}	G I Tract	7×10^{-3}
Fe^{55}	Blood	2	Blood	5×10^{-2}

taken into the body is t_1. The body irradiation continues for a time $t_2 - t_1$ after the intake is discontinued. The term X is given by the equation,

$$X = \frac{T_r - T}{T_r - T - T_r A + TB + T(A - B)(1 - C)} \quad \ldots\ldots (12)$$

in which $A = e^{-0.693\, t_1 / T_r}$, $B = e^{-0.693\, t_1/T}$ and $C = e^{-0.693\,(t_2 - t_1)/T}$. It is to be noted that the above equations take into account the reduction in concentration of the radioisotope in water or air during the period, t_1, that they are consumed; the radioactive and biological decay as deposition takes place in the body; and the continued irradiation of the critical body organ for a time $t_2 - t_1$, after the intake is discontinued. The values of Table II were obtained by the use of Equations 10 and 11. It should be observed from a comparison of Tables I and II that in some cases the critical organ may not be the same for a short period of exposure as for a long period. Diagram 2 shows the variation of dose rate with time in the case when water contaminated with I^{131} is consumed for 10 days and the initial concentration of the I^{131} in water is such that the total dose integrated over a period of 50 years is 25 rep. In this case it should be noted that most of the 25 rep is delivered to the thyroid in the first 40 day period. The equations do not take into account the possibility of selective absorption which might reduce the concentration of the radioisotope in the fluid during the period, t_1, of human consumption.

Under emergency conditions following the use of atomic weapons it may be useful to have a simple equation one can use to determine the

[1] These values were obtained by M. R. FORD of Oak Ridge National Laboratory using equations 10 and 11. Calculations were for a total of time of exposure $t_2 = 50$ years and an integrated dose of 25 rem. In each case the maximum permissible concentration was determined for several body organs and the lowest value was given in the table.

maximum permissible concentration of radioactive material in water and air as a function of the time since the atomic explosion. MORGAN and STRAUB (3) have suggested that the equation

$$(\mathrm{MPC})_{\mathrm{w,\,a}}^{\mathrm{emerg.}} = \mathrm{K}\ \mathrm{t}^{-1.2} \simeq \mathrm{K/t} \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (13)$$

can be used under such conditions. In this case $(\mathrm{MPC})_{\mathrm{w,\,a}}^{\mathrm{emerg.}} = \mu\mathrm{c/cc}$ of water or air. The equation can be used for the period from thirty minutes to a year following the explosion. If the time, t, is given in days, $\mathrm{K} = 10^{-3}$ for drinking water containing any contaminant emitting alpha, beta or gamma radiation. In the case of air contamination, $\mathrm{K} = 10^{-7}$ for beta or gamma emitting radioisotopes or 5×10^{-10} for alpha emitting radioisotopes. These values of K are sufficiently low to offer protection not only to the usual critical organ but also are expected to prevent damage to the gastro-intestinal tract and respiratory system during the early period of high concentration of the radioisotopes in water and air. The suggested value of K for air contamination is very low in order to reduce the hazard associated with radioactive dust particles.

The Particle Problem

The so called "particle problem" is concerned with the estimate of the biological hazard — if any — associated with insoluble dust particles of high specific radioactivity that may become lodged in human tissue — particularly in the lungs. Such particles may be generated in chemical operations or may result from an atomic explosion. The National (U. S.) Committee on Radiation Protection has not attempted to assign any maximum permissible concentration of such particles in the air and, until certain biological experiments now in progress are completed, one can only speculate on the hazards associated with such particles. One is concerned primarily with particles of about 1 micron diameter since larger particles are retained in the upper respiratory tract from which they are readily eliminated while the smaller particles are for the most part exhaled. If the size becomes so small (probably less than 1/4 micron) that the Brownian Movement is large, the lower respiratory retention increases again. However, since the particle activity usually decreases with the cube of the particle diameter — if one assumes uniform activity and a spherical shape — one is not as concerned about the submicron particles except where they are agglomerated by fagacitic action and fluid motion. The volume of tissue irradiated is quite small and the gram-rep dose is negligible but the dose rate in the vicinity of these particles may be many roentgens per day. MORGAN and STRAUB (3) have given equations and tables indicating the distribution of dose about these

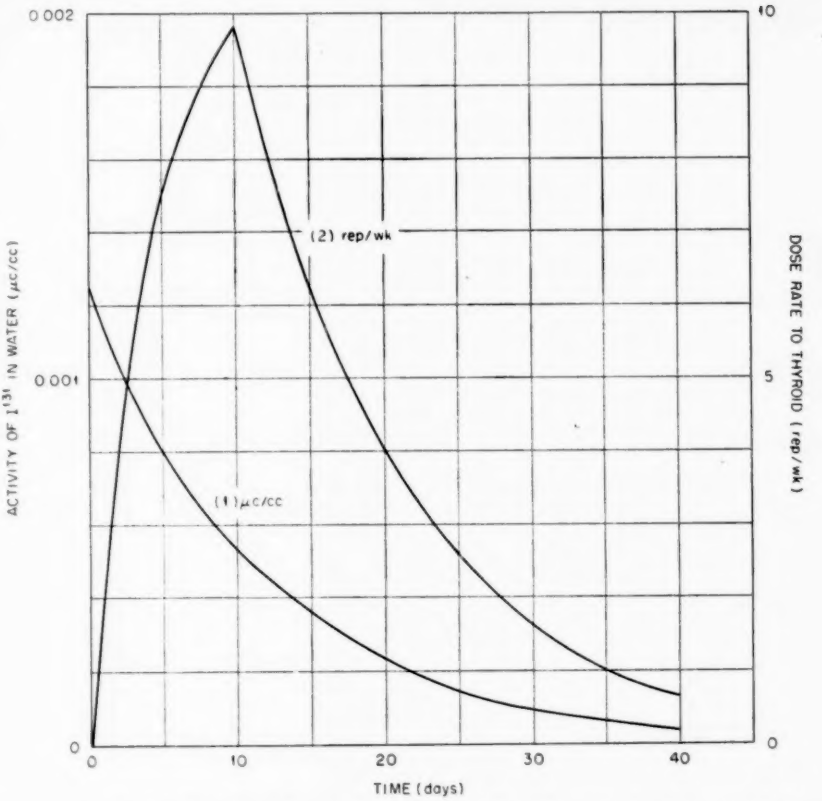

Diagram 2. Curve (1) gives the variation of activity of water due to normal decay of I^{131}. The initial concentration is such that if this water is consumed for 10 days the integrated dose to the thyroid is 25 rep. Curve (2) gives the dose rate to the thyroid as a function of time. The area under curve (2) for time $t_2 = 50$ years is 25 rep.

particles when they are fixed in tissue. The average dose to a sphere of tissue surrounding the particle when the radius of the sphere is greater than the maximum range of the alpha or beta radiation from the particle, is given by equation

$$D_{av} = \frac{13\ Q\ \Sigma(E_{eff})\ t}{X^3} \quad \ldots\ldots\ldots\ldots\ldots\ldots (14)$$

in which D_{av} = average dose in rep delivered in time, t days, Q = μc per particle, $\Sigma(E_{eff})$ = summation of effective energies of the radiation

Table III

Average Dose about a Radioactive Particle in Tissue.

Distance from Particle (cm)	Average Dose		
	From Particle of Constant Activity (rep)	From Particle of Decreasing Activity	
		$t_0 = 1$ day (rep)	$t_0 = 10$ days (rep)
10^{-4}	5.9×10^{4}	1.1×10^{4}	3.8×10^{4}
10^{-3}	6.0×10^{2}	1.1×10^{2}	3.9×10^{2}
10^{-2}	6.0	1.1	3.9
10^{-1}	5.1×10^{-2}	9.7×10^{-3}	3.3×10^{-2}
1	1.0×10^{-4}	2.0×10^{-5}	6.7×10^{-5}
10	1.0×10^{-7}	2.0×10^{-8}	6.7×10^{-8}

For values in this table it is assumed that the initial activity is from a 1 $\mu\mu$ curies particle; the radiation is from beta emitters with an effective energy of 0.8 Mev and the period of tissue irradiation is 10 days. The second column gives the average dose from a particle of constant specific activity. The third column gives the average dose from a mixed fission product particle that enters the body one day after the explosion. The fourth column gives the average dose from a mixed fission product particle that enters the body 10 days after the explosion.

from the particle in Mev and X = radius of sphere of tissue surrounding the particle in cm. For this case X must be taken greater than the maximum range of the radiation.

If one wishes to determine the average dose in the immediate neighborhood of a beta emitting particle and well within the maximum range of the beta radiation, it is given approximately by the equation,

$$D_{av} = \frac{13\ Q\ \Sigma(E_{eff})\ t}{X^3}(1 - e^{-\mu X}) \quad \ldots\ldots\ldots\ldots\ (15)$$

in which μ = coefficient of absorption of tissue for the beta radiation and X = radius of sphere of tissue in which X is taken less than the maximum range of the beta particle.

If the particle activity is due to mixed fission products resulting from an atomic explosion, the activity is continually decreasing and the two equations above must be multiplied by the term, F, given by

$$F = \frac{5\ t_0}{t}\left[1 - \left(\frac{t_0}{t_1}\right)^{0.2}\right] \quad \ldots\ldots\ldots\ldots\ (16)$$

in which t_0 = time since atomic explosion, at which the particle lodges in the body, t_1 = time since atomic explosion that the particle moves from its fixed position in tissue and thus $t = t_1 - t_0$ is the period of time the particle remains fixed in tissue. Table III gives the average

dose at various distances from a 10^{-12} curie beta emitting particle ($Q = 10^{-6}$ μc) that remains fixed in tissue for 10 days ($t = 10$). It is assumed that the particle is small enough to be treated as a point source with negligible self-absorption.

The dose close to the 10^{-12} curie particle is very large as indicated in Table III. Certainly many cells close to such a particle would be destroyed. It is those cells farther out from the particle that survive and undergo mitosis about which one perhaps should be concerned. There are many persons that do not consider this particle problem presents a serious radiation hazard. However, until one knows whether or not there is an appreciable increase in the probability of a tumor having its origin at the site of one of these radioactive particles, this will continue to be referred to as the "particle problem".

SUMMARY

Values are given of maximum permissible body burden, Q, and maximum permissible concentration, MPC, in air and water of some 70 radioisotopes. These values are published in a handbook prepared by the National (U. S.) Committee on Radiation Protection. In a few cases the values chosen are based on human experience, but in most cases they are calculated, using data from animal experiments. The value of Q is considered to be the microcuries of a radioisotope deposited in the total body that will deliver 0.3 rem per week to the critical organ, *i. e.*, the one receiving the radioisotope that results in the greatest over-all damage to the body. The MPC values are the concentrations of the radioisotopes in air or water that if used exclusively for an extended time will lead to an accumulation, Q, in the body. In most cases considered, equilibrium is reached in a few weeks. MPC values for air and water to be used in an emergency are given also. A large dose of ionizing radiation is delivered to tissue in close proximity to an airborne particle of high specific activity that becomes fixed in living tissue. In setting the MPC values for air one would like to know if such localized radiation increases the probability of tumor occurrence.

ZUSAMMENFASSUNG

Für etwa siebzig radioaktive Isotopen werden Werte für die höchstzulässige Körperbelastung, Q, und die maximal zulässige Konzentration, MPC, in Luft und Wasser angegeben. Diese Werte sind in einem Handbuch veröffentlicht worden, welches von der Nationalen (U. S.) Kommission für Schutz gegen radioaktive Bestrahlung vorbereitet worden ist. In einigen wenigen Fällen sind die angeführten Werte auf Erfahrungen an Menschen basiert, in den meisten Fällen jedoch sind sie nach Tierexperimenten berechnet worden. Unter *Q* wird die Menge eines radioaktiven Isotopen in Mikrocurie verstanden, welche das kritische Organ mit 0.3 rep/Woche bestrahlt. Unter dem kritischen Organ versteht man dasjenige, welches nach Aufnahme des radioaktiven Isotopen dem Körper den grössten Totalschaden zufügt. Die MPC-Werte geben die Konzentration der Isotopen in Wasser oder Luft an, die bei ausschliesslicher Benutzung während längerer Zeit zu einer Akkumulation, Q, im Körper führen. In den meisten beobachteten Fällen wird das Gleichgewicht in wenigen Wochen erreicht. MPC-Werte für Luft und Wasser zur Benutzung in kritischen Situationen werden ebenfalls angegeben. Eine grosse Dosis ionisierender Strahlung erhält das Gewebe in nächster Umgebung eines über dən Luftweg in ihm

fixierten Partikels von hohem spezifischen Gewicht. Für die Bestimmung der MPC-Werte für Luft wäre es wertvoll zu wissen, ob derartige lokalisierte Strahlung die Wahrscheinlichkeit einer Tumorbildung erhöht.

RÉSUMÉ

Les valeurs de la quantité maximum admissible d'isotopes radioactifs dans l'organisme, Q, et de la concentration maximum admissible (MPC) dans l'air et dans l'eau sont données pour environ 70 isotopes radioactifs.

Ces valeurs sont publiées par le Comité National des U. S. A. pour la Protection contre les Radiations dans un manuel en préparation. Dans quelques cas, les valeurs choisies sont basées sur l'expérience humaine, mais, dans la plupart des cas, elles ont été calculées à partir de données tirées de l'expérimentation animale. On considère que la valeur de Q est le nombre de microcuries de l'isotope radioactif contenu dans le corps entier qui délivre 0.3 rem par semaine à l'organe critique, c'est-à-dire à celui qui, après l'absorption d'isotope radioactif, cause le plus grand dommage à l'organisme entier. Les valeurs de MPC sont les concentrations en isotopes radioactifs de l'air et de l'eau dont l'usage exclusif et prolongé conduit à l'accumulation de la quantité Q dans le corps.

Dans la plupart des cas considérés, l'équilibre est atteint en quelques semaines. Les valeurs de MPC, pour l'air et pour l'eau utilisés en cas d'urgence, sont également indiquées. Les tissus situés à proximité immédiate d'une particule de grande activité spécifique, transportée par l'air et qui se fixe sur un tissu vivant, reçoivent une dose importante de radiations ionisantes. On aimerait savoir, pour la détermination des valeurs de MPC dans l'air, si cette irradiation localisée augmente la probabilité de survenue d'une tumeur.

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THE AMINES AND PARTICULARLY CYSTEAMINE AS PROTECTORS AGAINST ROENTGEN RAYS

by

Z. M. Bacq

I. Introduction

Thanks to the work of the radiochemists, it has become quite clear that the action of roentgen rays on aqueous solutions and on living organisms is mainly not a direct (target theory) but an indirect one through free radicals originating from the hydrolysis of water in the presence of oxygen (1, 10, 13, 23).

It is also proved beyond question that the action of roentgen rays on enzyme solutions and organisms can be partially inhibited by the presence of certain substances (DALE (23), BACQ (2), PATT (20)). Some substances, very active as protectors of enzymes in vitro, are also good protectors for animals; for instance: (a) thiourea for carboxypeptidase (DALE, 23) and for mice (MOLE, 13), (b) formate for carboxypeptidase (DALE, 23) and for mice (BACQ and HERVE, unpublished experiments).

The substances which were first shown to have in animals a protecting action against roentgen rays were toxic or had too weak an action; such was the case with thiourea, cyanide, malononitrile and azide (15), nitrite (12), estradiol (22, 26), cysteine and glutathione, and pyruvate (25, 6).

Another possibility of finding protectors is provided by the fact that the sulfur or nitrogen-mustards and roentgen rays have many actions in common. COLTER and QUASTEL (14), having shown that in vitro many amines protect choline oxidase against nitrogen mustard, we have systematically studied the effect of amines and of substances bearing the $-NH_2$ function.

II. Technique and Controls

Mice of pure breed (C57 black), about four months old and weighing from 19 to 21 g, are irradiated in groups of ten in a cardboard box (250 kv, 2.5 mm Cu, focal distance 50 cm, field 100 cm², 90 r per minute).

Lately, we have been using a Siemens Dosemeter in order to improve the accuracy of the dosage. The irradiated mice receive just the same attention as the controls; the surviving animals are counted every morning. Many controls have been made, and are run with each new series of investigated substances (5, 6). A single dose of 700 r kills practically all the controls. Out of 38 series of ten control mice, only seven were living on the 25th day after irradiation. This technique avoids the necessity of elaborate statistical analysis.

Equimolecular doses of substances (when toxicity permits), dissolved in 0.25 ml of saline, are injected intraperitoneally 1 to 4 minutes before the irradiation which lasts about six minutes. Twenty-five hundred mice were used in our work during the last eighteen months.

III. Results

Methylamine has a more marked protective action than ethylamine or ethanolamine. Aminoacids have, in general, a weak protective effect; this confirms previous work on microorganisms by HOLLAENDER and his associates. In the aromatic series, the amines are much more powerful than the corresponding aminoacids; and this is also true of histamine, cystamine and cysteamine. Tryptamine and 5-hydroxytryptamine (= serotonine = enteramine) seem of great interest but are toxic for mammals; we have injected one fourth only of the dose equimolecular to 3 mg of cysteamine (Table I). Various other — NH_2 bearing substances seem of little interest (Table II).

We have studied more extensively the two sulfur-bearing amines (6, 8) because it is known from the work of DALE and of PATT and coll. that sulfur (and particularly the — SH function) has a favourable action.

Cysteamine (= β mercaptoethylamine = 1,573 L = $HS - CH_2 - CH_2 - NH_2$)[1] is the simplest possible substance to carry simultaneously a free amine and a thiol function. Its interest is augmented by the fact that it is a fragment of pantotheine (or pantetheine = N-pantothenyl cysteamine) which is a growth factor for *Lactobacillus bulgaricus*

[1] This substance (with has been named Becaptan), cystamine and many other substances have been kindly synthetized for us by the Laboratoires de recherches pharmaceutiques de la Société Belge de l'Azote à Liège.

Table I

Number of mice (out of 10) surviving a dose of 700 r (lethal for controls) given after intraperitoneal injection of equimolecular amounts of amine and aminoacid. The doses are equimolecular to 3 mg β-mercaptoethylamine base, except in cases when toxicity is too great.

Amino-acid	Dose for 20 g mouse (mg)	Survivals (out of 10)	Amine	Dose for 20 g mouse (mg)	Survivals (out of 10)
Glycocolle	2.8	0	Méthylamine HCl	3.2	3—7
Alanine dl-*a*	3.6	3	Ethylamine HCl	3.9	1
l-*a*	3.6	2			
d-*a*	3.6	1			
Alanine β	3.6	0—2	Ethanolamine HCl	2	0
Serine dl	4	0		4.6	1—3
Cystéine l HCl	6.7	3	Cystéamine* = β-mercaptoéthylamine	3	10
Cystine (1/2)	4.7	0	Cystamine*	2.5—5	10
Valine l	4.45	1	Isobutylamine	2.8	?
Norvaline dl	4.45	1	n-butylamine	2.8	0
Leucine d	5.02	3	Isoamyline (HCl)	4.8	0
Leucine l	5.02	6			
Norleucine dl	5.02	1	n-amylamine	3.4	?
Lysine l (2 HCl)	8.6	0	Cadavérine	6.7	0
Phénylalanine	1.5	3	Phényléthylamine HCl	1.5	8
				3	2
			Phényléthanolamine HCl	3.4	4
Tyrosine l	1.7	0	Tyramine HCl	1.6	8
			Sympatol tartr.*	3.1	9
			Benzédrine SO_4*	1	0
			Ephédrine HCl*	1.9	1
Dioxyphénylalanine l (DOPA)	1.9	4	Oxytyramine HCl*	1.8	10
	7.5	0		7.25	7
			Artérénol (dl)* †	0.06—0.11	5—8
Tryptophane dl	2	2	Tryptamine	1.9	10
			5 hydroxytryptamine* (+ créatinine SO)	4.4	10
Histidine HCl	7.2	0	Histamine (see also refer. 16)	7	7
Proline l	4.3	6			
Asparagine l	5	4			
Ac. aspart. l	5.1	9			
Ac. aspart. dl	5.1	9			
a-amino-butyric dl	4	2			
Arginine l nitr.	9.3	1			
Ornithine l 2 HCl	7.8	4	Putrescine (2 HCl)*	6.1	0
Citrulline l	6.4	0			
Sarcosine	3.4	3			

* All the substances, except those marked * are Hoffmann-La Roche products.
? All animals dead from toxic action of amine.
† Noradrenaline.

(L. B. F.) and a fragment of coenzyme A, the importance of which is rapidly growing in biochemistry. It is not very toxic (LD/50 = 3.5 mg per 10 g weight) in mice. If a dose of 3 mg of cysteamine (base neutralized by HCl to about pH 7) is injected immediately before a dose of 700 r, ninety-seven per cent of the mice survive. In order to obtain about

Table II

Number of mice (out of ten) surviving a dose of 700 r given immediately after an intraperitoneal injection of various substances bearing one or more NH_2 functions. The doses are equimolecular to 3 mg cysteamine base when toxicity allows.

Substance	Dose for a 20 g mouse (mg)	Survivals	Substance	Dose for a 20 g mouse (mg)	Survivals
Aniline	3.5	1—4	Propylène diamine	3.1	9
Benzylamine*	4.1	1—3	Diaminodecane 2 HCl	0.9	0
Acide p-aminobenzoïque	5.1	0	Diaminodecane 2 HCl	1.8	1
Acide p-aminobenzoïque	10.2	1	Spermine*	1.9	0
Sulfanylamide	1.6	0	Spermidine*	2.1	0
Acide p-aminosalicylique	5.8	0	Taurine*	4.8	1—2
Allylamine*	0.9	0	Taurine*	9.6	0
Hydroxylamine	1.3	0	Pantoyltaurine*	9.9	0—3
Diméthylamine HCl*	3.1	3	Pantoyltaurine*	19.8	8
Triméthylamine HCl*	3.6	3	Créatine*	5.7	0—2
Diéthylamine*	4.2	0—8	Créatine*	13.9	2
Diéthylamine*	8.3	0	Créatinine*	4.3	0
Di-β-oxyéthylamine*	4.4	0	Agmatine*	8.6	5
n-Propylamine HCl	6.2	0	Gramine*	1.7	4
Ethylène diamine 2 HCl	4.2	3	Arcaïne*	2.5	0

* All these substances are Hoffmann-La Roche products (Basel).

the same mortality curve as that of the controls, one must give at least 1,300 r (Fig. 1).

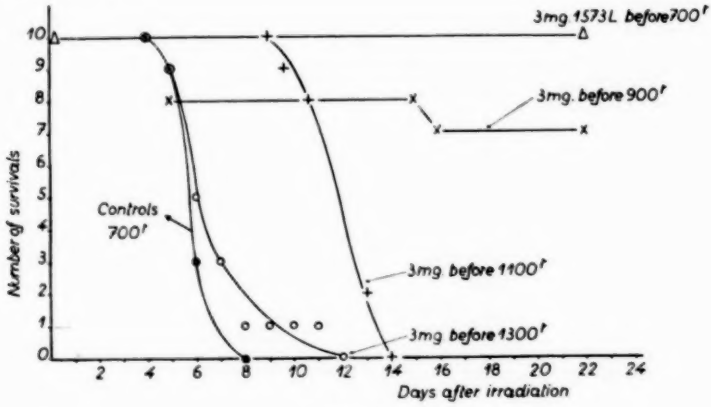

Fig. 1.

Abscissae: days after a single irradiation.
Ordinates: number of survivals of five series of ten mice.

If the injection is made immediately after irradiation (700 r) the mortality curve is identical with that of the controls. These facts have been confirmed by PHILPOT (Harwell) and by MAISIN (Louvain, Belgium) in mice and by MANDEL (Strasbourg) in rats.

Cystamine $\begin{Bmatrix} S-CH^2-CH^2-NH^2 \\ | \\ S-CH^2-CH^2-NH^2 \end{Bmatrix}$ is at least as good as cysteamine (6); it liberates large amounts of histamine, and this fact prevents its use in human therapeutics (17). It is reduced to cysteamine in vitro by cysteine, and also in vivo probably by glutathione and other — SH bearing protides.

A very simple experiment shows how weakly reducing is cysteamine: at room temperature and at neutral pH, cystamine oxidizes cysteine and a precipitate of cystine is obtained. It is possible that one of the main functions of glutathione and other — SH substances in the body, is to keep coenzyme A in the reduced state. Oxidized CoA (— S — S —) is inactive because the exchanges of acetyl radicals are situated on the thiol function.

Cysteamine when injected is rapidly metabolized; it has disappeared from the blood of dogs and rabbits fifteen minutes after the intravenous injection of large amounts and has been excreted unchanged in the urine during the period. Its protective action (and also that of cystinamine) against roentgen rays is of short duration; one hour after the injection, the radiosensitivity of the mice (tested by the mortality after 700 r) has come back to normality (6).

Cysteamine also induces a long-lasting (at least five hours) liberation into the blood (rabbit) and urinary excretion (dog and man) of reducing substances, one of which is ascorbic acid (4). The ascorbic acid content of the adrenal glands of the rat falls to half of the normal value three hours after the intraperitoneal injection of cysteamine (10 mg for 100 g rat). This is not accompanied by a 'stress' reaction (fall in the circulating eosinophiles and of cholesterol content of the adrenal gland and increase of urinary excretion of steroids). This reaction is thus very different from that of ACTH (adrenocorticotrophic hormone), salicylates, etc. . . . (27); it is not given by cysteine. We do not know if this remarkable property is linked with the protective action of cysteamine and its effect on radiation sickness (see below).

Cysteamine acts directly on cells; it protects against roentgen radiation and ciliates (9) the reticulocytes of dog's blood in vitro (19) and growing roots of peas (6). Both cysteamine and cystamine partially inhibit the oxidation of ferrous sulphate by roentgen rays (BACQ, Z. M. and PIROTTE, M., 23).

Cysteamine has marked antimitotic properties for muscle cells of

chick embryo in tissue culture; its action seems mainly on the protoplasm, not on the nucleus (11). In some cases of human chronic leukemia, cysteamine has given good results (3).

IV. Cysteamine in Radiation Sickness

Relatively small doses of cysteamine (200 mg injected intravenously at any time except one hour before irradiation) are well-tolerated and active against radiation sickness in cancer patients treated by radiotherapy. Diarrhoea, vomiting, nausea and general weakness disappear after one or two injections; three doses are rarely needed (16). We can testify that it does not decrease the beneficial effects of the radiotherapeutic treatment. Cysteamine is now systematically used in the Department of Radiotherapy of the University of Liège.

The apparent contradiction between these clinical observations and our results in mice, is interesting and easily explained; it is not a paradox.

1) Radiation sickness in patients irradiated locally and mortality of mice which have received a lethal dose on the whole body, are different phenomena.

2) Cysteamine (or cystamine) protects mice against death but this does not mean that all the actions of the roentgen rays are inhibited: for instance, the surviving females are sterile; their ovaries are completely destroyed (15) and the hair becomes grey. Experiments are in progress to investigate how the reactions of normal and cancerous tissues to roentgen radiation are changed by the presence of cysteamine.

3) The fact that cysteamine does not change the mortality curve if injected in mice immediately after irradiation, does not mean that it has no action. Recent unpublished observations of MAISIN (Louvain, Belgium) are of great theoretical and practical importance. Rats injected with mercaptoethylamine after a dose of 1,000 r die rapidly; rats which receive 1,000 r with the liver protected by lead, also have a high mortality rate, but if such irradiated rats (with protection of the liver) receive three injections of cysteamine after a dose of 1,000 r, the mortality is strikingly reduced. Thus, there seems to be a curious synergy between liver protection and cysteamine action. This synergy might be explained by the fact that the liver is the main source of the ascorbic acid liberated by the drug injection (24), and that ascorbic acid seems to play a rôle in the biochemical lesion resulting from roentgen irradiation (18).

Conclusions

We have tested the protecting effect against 700 r total body irradiation (treshold ninety-eight per cent lethal dose) of equimolecular doses

(when toxicity permitted) of forty-five amines injected intraperitoneally. At least ten mice (C57 black) were used for each test. Seventeen did not change the mortality curve; twenty-two gave at least three survivals. The most active amines are β mercaptoethylamine (or cysteamine = $HS-CH^2-CH^2-NH^2$) and its $-S-S$ derivative, tryptamine, hydroxytryptamine, oxytyramine, noradrenaline and histamine. These amines are, at equimolecular doses, more active than the correspondent aminoacids.

Cysteamine has been studied with special care because of its low toxicity (L. D. 50 = 350 mg/kg) and because it is a physiological molecule a fragment of pantetheine (L. B. F. growth factor) itself a fragment of coenzyme A.

Cystamine ($-S-S$), because it is a diamine, is a powerful histamine liberator and cannot be used in human beings, but its protective action against roentgen rays is theoretically important because cystine (the corresponding aminoacid) is inactive. Cystamine when injected intravenously in dogs and rabbits is reduced to cysteamine.

3 mg cysteamine per 20 g mouse (150 animals) injected immediately before 700 r gives ninety-seven per cent permanent survival (while ninety-eight per cent of 250 controls die). In order to obtain the same mortality curve in cysteamine injected animals as in controls, one must increase the dose to about 1,300 r.

If cysteamine is injected immediately after irradiation, the mortality curve is the same as that of the controls. The radioprotection is of short duration; the molecule is rapidly metabolized; one hour after the injection the radiosensitivity has come back to about normal.

In cancer patients treated with large doses of roentgen rays, one or two intravenous injections of 200 mg cysteamine suppress post-radiation symptoms.

Addendum (Dec. 1953)

It has recently been found that cystamine *per os* is an outstanding protector for mice, and that 800 mg are very well tolerated when ingested by man.

This work has been performed with the collaboration of A. Herve, P. Fischer, J. Lecomte, Miss M. Pirotte, of the University of Liège, G. Dechamps, Le Bihan and P. Rayet, of the Lab. Rech. Pharm. Soc. belge Azote, Liège.

The expenses have been covered in great part by grants from the Ministère de l'Intérieur, Conseil Supérieur de la Sécurité Civile.

SUMMARY

A series of investigations into the protecting effect of equimolecular doses of a large number of amines against 700 r total body irradiation in mice is reported.

The importance of cysteamine, an amine of low toxicity, as a protective agent is discussed in detail. It has been found that relatively small doses of cysteamine will suppress radiation sickness following radiotherapy in human subjects.

ZUSAMMENFASSUNG

Es wird über eine Reihe von Untersuchungen über die Schutzwirkung von equimolekularen Dosen einer grossen Anzahl von Aminen gegenüber 700 r Totalbestrahlung von Mäusen berichtet.

Die Bedeutung des Cysteamins, eines Amins von geringer Toxizität, als Strahlenschutzmittel wird eingehend besprochen. Man hat gefunden, dass verhältnismässig kleine Dosen von Cysteamin den Röntgenkater im Anschluss an die Strahlenbehandlung des Menschen verhindern.

RÉSUMÉ

L'auteur rend compte d'une série de recherches sur l'effet protecteur de doses equimoléculaires d'un grand nombre d'amines contre l'irradiation par 700 r de tout le corps chez la souris.

Il examine de façon détaillée l'intérêt de la cystéamine, amine peu toxique, comme agent protecteur. Il a trouvé que des doses relativement faibles de cystéamine sont capables de supprimer le mal des rayons consécutif à la radiothérapie chez l'homme.

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FROM THE INSTITUTE OF RADIOPHYSICS (DIRECTOR: PROFESSOR ROLF SIEVERT), STOCKHOLM, SWEDEN

SOME REMARKS ON CHEMICAL PROTECTION AGAINST IONIZING RADIATION

by

A. Forssberg

Protection against ionizing radiation by chemical means may apparently be reached in a variety of ways. The diversity of substances successfully tried indicates that the explanation of the underlying mechanism probably must be sought along various lines. BACQ has told us about investigations of the application of amines and cysteamines, and GRAY dwelt, among other things, upon the effect of oxygen pressure. HOLLAENDER in his lecture at the Ciba Conference two years ago mentioned a number of widely different substances, which were protective on bacteria.

In collaboration with N. NYBOM I have investigated the protective effect of cysteine on *Allium* roots, primarily with a view to correlate the survival and growth rate data with cytologic features as well as with biochemical events which eventually may preceed or accompany the biologic effects. Some results concerning parallel determinations of growth rate and survival and the cytology of the cell may be of interest in connection with the matter discussed at this conference.

Moderate doses of roentgen rays (100—200 r) caused a transient growth depression of the roots with a minimum of growth rate six to eight days after the irradiation. The ratio of the growth rate of cysteine treated and irradiated roots to roots irradiated in the usual way (in water) averaged two to three at that time. The number of total chromosome disturbances (bridges, fragments) was at the same instance two to three times higher in the last mentioned group of roots. Our results agree fairly well with the experiments of GRAY, READ, THODAY and others on *Vicia* roots, irradiated in oxygen as well as in nitrogen, and indicate a certain resemblance in the effect of cysteine and of anaerobiosis. It may be added that we, too, found less protection against α rays than against roentgen rays from cysteine as did the authors mentioned, with nitrogen. In a special *Allium* strain we found by chance that the protective effect of cysteine

was much less pronounced than usually, around 1.5 at the most. The same strain showed no significant difference in the percentage of chromosome disturbances between the two root groups. This fact adds further evidence to the correlation between the degree of protection and chromosome damage.

High doses (700—800 r) invariably killed roots irradiated in water while cysteine protected roots survived to some 60 to 70 per cent. In spite of the pronounced difference in survival the percentage difference in the number of chromosome disturbances was, however, quite insignificant at this high dose. In an actual example we obtained, for example, 65 per cent survival of cysteine roots against nil of water roots, while the chromosome damage was only eleven per cent higher in the water roots than in the latter at the time of maximal disturbance of the mitotic process. It seems to us unlikely that such a small difference in chromosome damage could account for the much better survival of the cysteine roots in this example.

The two root groups in this experiment differed, however, from each other with respect to the rapidity of the passage through the amitotic period which follows upon irradiation. The mitotic frequency relative to unirradiated controls reached a peak value of ninety-five per cent already forty-eight hours after irradiation in the cysteine treated groups against a peak value of ninety-two per cent, ninety-six hours after the irradiation in the water groups. Thus, neither the absolute numbers of reappearing mitosis nor the number of chromosome disturbances differed very much in the two groups, but the time of the recovery period was shortened in a very significant way after cysteine treatment. This indicates that processes of a biochemical nature are presumably effective in the restoration of irradiation damage. It may be added that cysteine *per se* (*i. e.* without any irradiation) slightly depresses the growth rate for a few days after the application, *i. e.* acts in a way which is quite opposite to the combined effect of roentgen rays and cysteine compared with roentgen rays given in the ordinary way (in water). The growth depression of cysteine is a transient effect which cysteine has in common with almost every other chemical substance, at least at a certain concentration level, *e. g.* other amino acids which in spite of this do not protect. It thus seems that the explanation cannot plainly be that such an unspecific depression of the growth processes at the time of the irradiation affords protection.

SUMMARY

Cysteine protects *Allium* roots against damage from roentgen rays with a factor of ~ 2. Chromosome disturbances (fragments, bridges) were reduced to 50 percent or less in the cysteine treated roots. The action of cysteine was also manifest from a more rapid repair of the roentgen damage.

ZUSAMMENFASSUNG

Cystein schützt Alliumwurzeln gegen Schäden durch Röntgenstrahlen mit einen Faktor ~ 2. Chromosomenstörungen (Fragmentierungen, Brückenbildungen) wurden bei den mit Cystein behandelten Wurzeln auf 50 % reduziert. Die Wirkung der Cystein zeigte sich auch in einer schnelleren Erhohlung nach Röntgenschäden.

RÉSUMÉ

La cystéine protège les racines de *Allium* contre les lésions dues aux rayons roentgen avec un facteur ~ 2. Sur les racines traitées par la cystéine, les atteintes chromosomiennes (fragments, ponts) ont été réduites à 50 pour cent ou moins. L'action de la cystéine s'est aussi manifestée par une réparation plus rapide des radiolésions.

REFERENCE

A full account of this work is given in: A. Forssberg and N. Nybom: Combined effects of cystein and irradiation on growth and cytology of Allium cepa roots. Physiologia Plantarum 6 (1953), 78.

ON CHEMICAL PROTECTION AGAINST IONIZING RADIATION

by

Z. M. Bacq

Our results with mice and cysteamine are very similar to those of FORSSBERG with onion and cysteine. We have seen that in mice protected by cysteamine, the primary radiation damage occurs just as in the controls: for instance, during the 24 hours after irradiation, the weight of the protected animals falls (fig. 1) and the white cell count

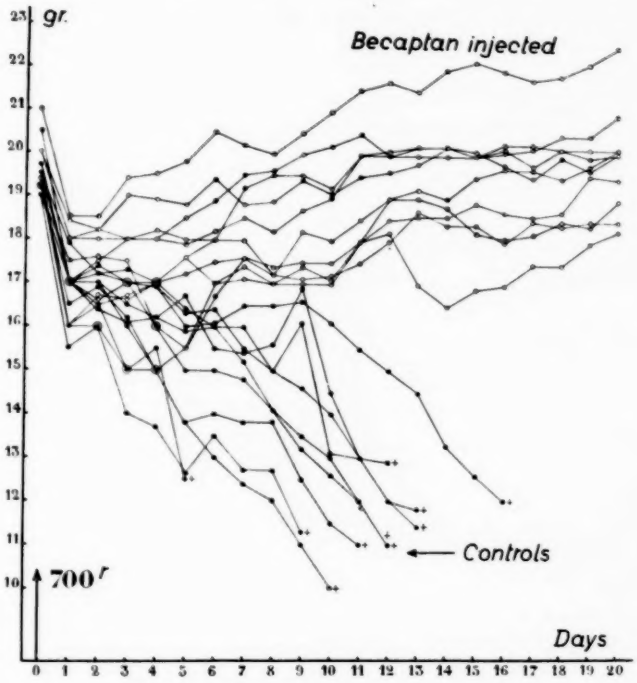

Fig. 1.

Ordinates: weight in grams of 20 mice, ten of which have been injected with cysteamine (3 mg intraperitoneally) before irradiation (700 r), ten others serving as controls.

Abscissae: days after irradiation.

All the animals loose weight during the first day, there is progressive recovery of the injected animals (which survive) while the weight of the controls drops until death.

is lowered just as in the controls. But this decrease in weight is halted as early as 48 or 72 hours afterwards and an increase of weight begins in the injected animals. The weight of the controls drops until death. It seems that the presence of cysteamine during irradiation has protected some regeneration factor (possibly, coenzyme A). The white cell count of irradiated protected mice begins to rise (*i. e.* regeneration of these cells becomes active) much later, (on the 8th day if 550 r are given, on the 10th day if 700 r are given) but earlier than the controls when they survive. On the other hand, there is no regeneration of the ovaries, and the adrenal cortex remains much thinner (DESAIVE, BACQ and HERVE). Thus each tissue seems to be affected by irradiation whether cysteamine is present or not; it is the regeneration, the reactions of the organism against radiation damage, which is enhanced when cysteamine is present. The various tissues of mammals have very different regeneration possibilities.

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FROM LABORATOIRE PASTEUR DE L'INSTITUT DU RADIUM, PARIS, FRANCE, AND MEDICAL RESEARCH COUNCIL, RADIOTHERAPEUTIC RESEARCH UNIT, HAMMERSMITH HOSPITAL, LONDON, ENGLAND

DEFINITION OF THE TERMS 'PROTECTION' AND 'RESTORATION'

by

R. Latarjet and *L. H. Gray*

In order to clarify our thought regarding the influence of environmental factors on radiobiologic damage, it is convenient to consider, quite generally, four stages in the development of any particular observable change in a cell (tissue, organ, or organism), resulting from exposure to radiation, which we refer to as the 'observable lesion'.

1		2		3		4
Absorption of radiant energy	→	Primary radiochemical reactions	→	Chemical reactions chains	→	Observable lesion
		latent period				

In the first step, the distribution of the absorbed energy within the cell is determined by the chemical structure of the cell and the type of radiation to which it is exposed. The absorbed energy will be distributed among the various possible modes of vibration of the molecules within a time of the order of 10^{-13} sec., to be followed by the second step which is, in most cases, probably complete in times of the order of 10^{-9} sec. At this stage, part of the energy is in the form of very reactive and very short-lived radicals, which in turn give rise to intermediates of longer life, and to reversible and irreversible chemical changes, which we have designated as the third step. Steps 2 and 3 together comprise the latent period, and it is probable that in many cases the course of the changes which take place during steps 2 and 3 will be affected by the chemical composition of the living protoplasm. Those changes which constitute step 3 will also be conditioned by the active metabolism of

the cell, and therefore by environmental factors such as temperature and nutrients.

We propose to use the term 'protection' to describe a diminution of the observable lesion resulting from a treatment or procedure which is operative before the beginning of irradiation, and the term 'restoration' when this is achieved by treating the cell in some way after the beginning of irradiation. The term restoration thus includes procedures hitherto described by such terms as photoreactivation and heat-reversal, and also covers control of the natural process of recovery. We have chosen to define the terms protection and restoration in the manner indicated so as to descriminate unambiguously between a successful pre-treatment and a successful post-treatment. They are not to be understood as carrying any particular connotation regarding the mechanism by which the development of the lesion is modified. Indeed, it may well be that restoration is more often achieved by blocking the development of the lesion at some point in the latent period than in the restoration of the precise chemical condition of the cell prior to irradiation. It will be noted that we are considering always the protection or restoration of a particular observable lesion, and not of the cell as a whole. For example, mutations may persist in a cell in which the ability to divide has been restored.

The general observations on the development of radiobiological lesions and the definition of the terms 'protection' and 'restoration' described in this paper will be used in the following two papers.

FROM THE MEDICAL RESEARCH COUNCIL, RADIOTHERAPEUTIC RESEARCH UNIT,
HAMMERSMITH HOSPITAL, LONDON

CONDITIONS WHICH AFFECT THE BIOLOGIC DAMAGE RESULTING FROM EXPOSURE TO IONIZING RADIATION

by

L. H. Gray

Introduction

The distribution of energy within cells exposed to ionizing radiation is, of course, determined by the type of radiation employed. For a given flux of radiation of given type, the amount of ionization and excitation produced along the particle tracks, and the relative frequency with which particular molecular groupings are directly affected by the ionizing particle, are essentially unalterable by any means at our disposal.

The absorbed energy will be distributed among the various possible modes of vibration of the molecules within a time $\sim 10^{-13}$ seconds, to be followed by dissociation and chemical reaction within $\sim 10^{-9}$ seconds. Part of the energy at this stage is in the form of very reactive and very short-lived radicals, which in turn give rise to intermediates of longer life, and to reversible and irreversible chemical changes. It is at this point that we are first able to exert some control over the development of a radiobiologic lesion by modifying the environmental conditions.

An overriding factor which determines the reactions to which the short-lived radicals give rise is the disposition of these radicals along the tracks. If they are formed very close together, they react among themselves in a manner quite different from that when they are more sparsely distributed, and this profoundly affects the chemical and biologic changes which follow. Examples are shown in Fig. 1 (GRAY, 1953). No chemical treatment at the time of irradiation, which has so far been tried, has been found to modify appreciably damage induced by the high ion density radiations. (GRAY, 1952.)

Among the earliest quantitative studies of the influence of environment on radiosensitivity were those of HOLTHUSEN, who in 1921 showed that it was necessary to expose *Ascaris* eggs to three times as large a dose of roentgen radiation under anaerobic as under aerobic conditions, in order to prevent a fixed proportion of the eggs from developing into normal worms. During the intervening thirty years, the influence of anoxia on many aspects of radiobiologic damage has been studied. In addition there are many papers reporting a reduction in radiosensitivity — or, as we may prefer to look upon it from the standpoint of this Conference, a measure of 'protection' — which has been achieved by procedures, including interference with blood supply, which might be expected to reduce the oxygen tension in the tissues at the time of irradiation. It will be most convenient to consider first those experiments bearing on the rôle of dissolved molecular oxygen.

The Influence of Dissolved Molecular Oxygen

Radiobiological Damage in General

It has been established for many diverse types of radiobiological damage that the effectiveness of a given dose of roentgen radiation is conditioned by the concentration of oxygen dissolved in the cell at the time of irradiation. In all forms of damage so far investigated, the length of the pre-treatment period which gives maximum protection is only that necessary to establish the changed oxygen tension in the tissue under examination, and I have found only one instance out of a very large number — viz., in respect of inhibition of division and cell degeneration produced by whole-body roentgen irradiation in the haemopoietic tissue of bullfrog tadpoles (ALLEN *et. al.*, 1951) — in which a change in oxygen tension for a short period immediately following irradiation has been reported to influence radiosensitivity. In the exceptional case referred to, the change in oxygen tension after irradiation may have been effective by altering the rate of development of the cells, and therefore the time of appearance rather than the magnitude of the damage. In similar studies of radiation damage induced in the bone marrow cells of mice, DEVIK (1952) finds a marked effect of pre-irradiation anoxia, but no effect of post-irradiation anoxia, in conformity with all other observers.

In favourable materials, *e. g.*, microspores, in which oxygen tension may be changed rapidly, it has been shown (GILES and RILEY, 1949 and 1950) that the degree of chromosome damage induced by a given dose of radiation is sensibly independent of changes in oxygen tension which occur more than half to one minute before or after irradiation.

Following upon the experiments of TITUS EVANS and of LACASSAGNE

in 1942, it has been shown by a number of workers that mice and rats survive whole-body exposure to larger doses of radiation if delivered under hypoxia or anoxia, and that specific types of damage, *e. g.*, damage to the bone marrow, are similarly affected. Frogs are not exceptional, as at first appeared to be the case (PATT, 1952). Marked protection with respect to lesions of the skin and hair follicles (EVANS, 1942), to the regression of an inoculated mouse tumour (HOLLCROFT, LORENZ, and MATTHEWS, 1952) irradiated *in vivo*, has also been demonstrated. We do not yet know whether different lesions in the same animal are affected to the same extent by a given degree of hypoxia.

The citation of the percentage of animals which survive under two different experimental conditions gives no quantitative measure of the effectiveness of a particular treatment, which can clearly only be stated numerically if the dose-response curve is known to be the same under both experimental conditions. For this and other reasons, special caution is necessary in evaluating the effectiveness of a given treatment upon survival. It is, however, probably safe to say that severe hypoxia during irradiation provides rather greater protection to animals than any other form of pre-treatment so far investigated. In the case of the irradiation of pregnant female mice, the foetus is protected to about the same extent as the mother. It has been shown, moreover, by LIANE RUSSELL (1952) that the protection is about the same for a number of foetal injuries induced by irradiation on the eleventh day of gestation, namely, tail length, tail shape, and fore and hind foot structure. Hypoxia induced by breathing 5 % oxygen with 95 % helium was equivalent to a dose reduction factor of 1.6.

As regards damage induced by roentgen radiation, in all cases so far investigated the effect of hypoxia appears as a numerical reduction in the efficacy of a given dose of radiation, which is about the same regardless of the absolute magnitude of the dose over the whole range from 50 r to 100,000 r. At the one extreme, this has been observed to hold for the arrest of cells about to enter prophase in both grasshopper neuroblasts (GAULDEN and NIX, 1950) and plant meristems (READ and WOLFF, 1948), and at the other for the disinfection of bacteria. The bacteria studied by HOLLAENDER, STAPLETON and MARTIN (1951) presented an interesting situation in that clones may be obtained of very different aerobic sensitivity by changing the culture conditions during the preceding 24 hours. The inactivation of one clone is exponential, and of another markedly sigmoid. Irradiation under anaerobic conditions leaves the shapes of the inactivation curves unchanged, but reduces the effectiveness of a given dose by a factor of 3.

TROWELL, (1953. I am indebted for permission to report these results in advance of publication) has observed a somewhat larger effect

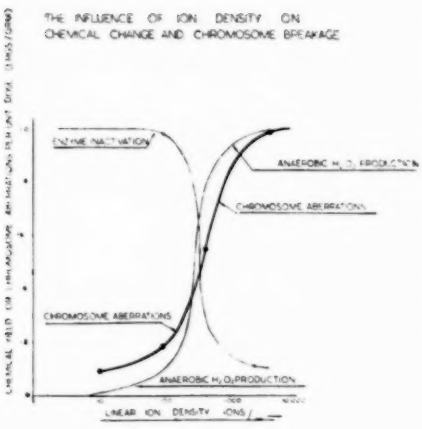

Diagram 1.

of oxygen tension than I have found recorded previously in the literature. He has studied the degeneration of small lymphocytes from the nodes of young rats cultured and irradiated in vitro. Degeneration in these cells sets in in a matter of a few hours after irradiation and is not associated with mitosis. In terms of his criteria of death, the dose survival curve was not quite identical under aerobic and anaerobic conditions. According to the level of survival which is considered, the effect of irradiation in nitrogen compared with that in pure oxygen was a dose reduction factor of between 8 and 12.

These examples suffice to show the generality of the protection afforded by anoxia. They are most naturally interpreted in terms of a diminution in the number of effective chemical intermediates produced per unit expenditure of energy along the tracks of the secondary electrons generated by the roentgen radiation. This interpretation receives strong support from the close similarity between the curves relating oxygen concentration to the 'protective factor' in a number of different tissues. (I use the term 'protective factor' for the ratio of the doses required to produce identical biologic effects under two different conditions of oxygen tension.)

Figure 2 gives five examples of the influence of oxygen tension on radiosensitivity. Full lines in all cases give the original experimental data as presented by each author. Broken lines show estimates of relative sensitivity by the writer. The reciprocal of the dose required to produce a specified biological reaction is taken as the true measure of relative sensitivity. These are respectively:

(a) Growth inhibition in *Vicia faba* roots (Read, 1951).

(b) Chromosome interchanges produced in *Tradescantia* microspores (Giles and Beatty, 1950).

(c) Sex-linked lethal mutations induced in the sperm of *Drosophila melanogaster* (Hollaender, Baker and Anderson, 1951).

(d) Translocations between autosomes of *Drosophila virilis* (Hollaender, Baker and Anderson, 1951).

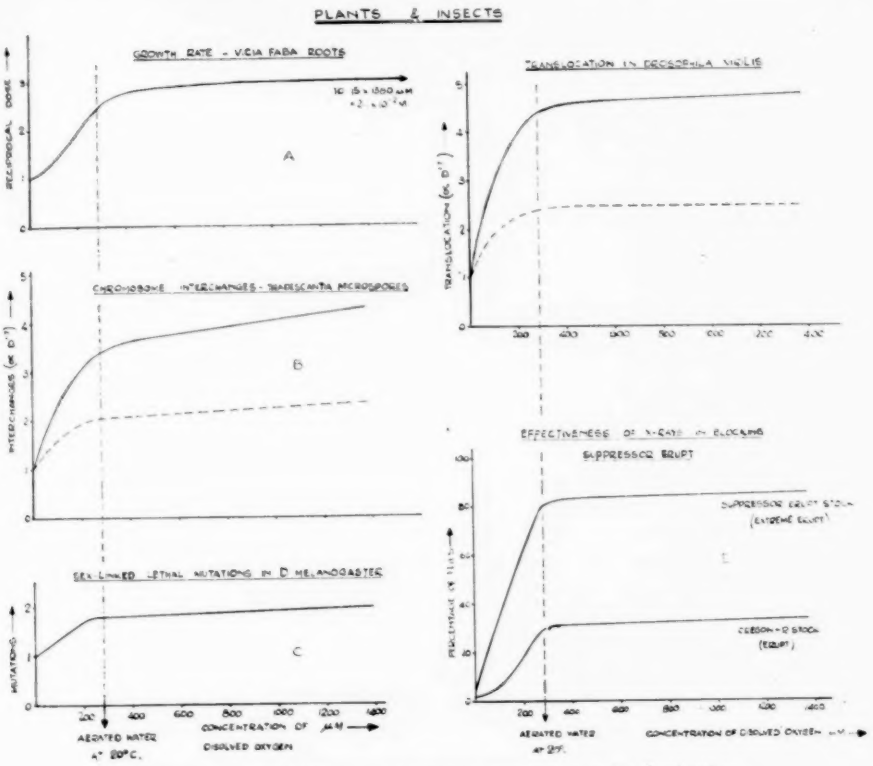

Diagram 2. The influence of oxygen tension on radiosensitivity.

(e) The effectiveness of roentgen rays in blocking the action of a suppressor gene in *Drosophila melanogaster* (GLASS and PLAINE, 1952).

We are not yet in a position to enter at all deeply into the interpretation of these curves. It may be said, however, (1) they appear to indicate a common chain of chemical events initiated by the ionizing radiation and responsible for the different biologic changes; (2) under normal conditions of aeration a large part of the biologic effect in each case involves a chemical chain which at one point includes reaction with molecular oxygen; (3) that in terms of Weiss's hypothesis (1944) the greater part of these particular biologic effects produced under aerobic conditions would be attributed to H., through the intermediary of HO_2, and the residue to OH·. While there are very good reasons for believing that many radiochemical oxidations, and some inactivations, including the

inactivation of certain enzymes (COLLINSON et al., 1950) are attributable to the HO· radical, there are no equally compelling reasons for implicating this radical as the initiator of radiobiologic damage.

It might be argued that since it has been shown, *e. g.*, by RAJEWSKY and his colleagues (1952), that the effectiveness of a given dose of roentgen radiation is reduced by about a factor of 3 when spores are irradiated at liquid air temperature instead of at room temperature, the origin of the residual effect should be looked for in directions other than in the medium surrounding the biologic structures, since no diffusion of radicals can take place at liquid air temperatures. I think this is not necessarily the case. The actual biologic damage is only observed when the spores are returned to room temperature, and it has been demonstrated, *e. g.*, by ZIMMER [1944, see G. STEIN, Faraday Soc. Trans (in press)] with respect to the reduction of methylene blue by roentgen radiation, that energy may be trapped and stored at liquid air temperature, to be used to produce radiochemical change when the solution is returned to room temperature.

An explanation of the protection afforded by anoxia against roentgen damage in terms of a different utilization of the radicals formed along the electron tracks receives additional support when we consider the relative absence of protection afforded by anoxia in the case of densely ionizing radiations such as α-radiation and neutron radiation.

The observations of READ and THODAY (1947 and 1949), (READ, 1952a) on growth inhibition and chromosome damage in *Vicia* are well known. In a recent paper, READ (1952b) has recalculated the protection afforded by anoxia in the case of α-radiation as about 20 %, *i. e.*, a protection factor of 1·2.

During this year Mr. J. W. BOAG and I have studied the influence of anoxia on growth inhibition in *Vicia* roots induced by 3·6 MeV neutrons. These experiments (to be reported fully elsewhere) have indicated a protection factor close to that observed by READ and THODAY for α-radiation, viz. about 1·4. This is, in any case, in very marked contrast to the factor of 2·6 observed with roentgen radiation, but when we allow for the fact that in tissues exposed to α-radiation and neutron radiation, part of the ionization is produced by δ-rays which travel clear of the central dense column of ionization, it would appear that within that column, the chemical changes are sensibly independent of the presence of molecular oxygen at the concentrations studied so far, in agreement with the chemical evidence of BONET-MAURY and LEFORT (1950), (and BONET-MAURY, 1952).

In other studies which we have made of the modification of biologic damage by chemical means, to which I shall refer later, neutron radiation also showed itself much less subject to environmental influence than roentgen radiation.

Genetic Damage

We have already considered several examples of the influence of anoxia on chromosome damage induced by roentgen radiation. Such influence is firmly established for germinal and somatic cells in *Vicia*, *Tradescantia*, barley, maize, *Drosophila*, and mice. I find the position much less clear with regard to other forms of genetic damage. I see no reason why the damage resulting from the ionization of a complex molecular structure such as a gene should necessarily be independent of its chemical environment. It is noteworthy, however, that HEWITT and READ (1950) observed the direct inactivation of one of the T series of *E. Coli* bacteriophages, which is believed to follow from the ionization of the substance of the virus, to be uninfluenced by anoxia.

W. L. RUSSELL (1951) has observed that the number of dominant lethal mutations induced in mice which were breathing 5 % oxygen for a short period before and during exposure to radiation was not different from the number induced under normal aerobiosis. Measurements of the actual oxygen tension which prevails in tissues of animals receiving such treatments are badly needed, but in this case it may be assumed that a condition of hypoxia existed in the germinal cells, since the effect of roentgen radiation on the length of the sterile period (as well as on lethality and the growing of hair) was reduced by the treatment.

In micro-organisms, HOLLAENDER, BAKER and ANDERSON (1951) found only a slight influence of oxygen tension on the induction of the "mutation" in *E. Coli* from streptomycin dependence to streptomycin independence. In contrast with this, they find a very marked effect on the reverse mutation from purine dependence to purine independence. Two points about the purine curves are noteworthy: (1) the mutation frequency is very far from linearly related to the dose, and (2) the influence of oxygen tension on mutation rate exactly agrees with that on lethality. It has been shown (GRIGG, 1952) that the growth of normal organisms can be prevented by plating with a large excess of biochemically deficient organisms, and that relief of the population pressure through death can lead to the appearance of mutants. With this in mind, we should perhaps consider the exact parallelism between the effect of oxygen on lethality and the mutation frequency to purine independence as a reason for suspending judgment until the possible influence of population pressure has been ruled out. (In a private communication Dr. ANDERSON tells me he has examined this point and believes that the observed effects are not influenced by population pressure.)

Two other cases have so far been recorded in the literature in which hypoxia has been found to reduce the rate of induction of mutations by roentgen radiation, namely, (a) the induction of sex-linked lethal

mutations in *Drosophila* (BAKER and SGOURAKIS, 1850, and HOLLAENDER, BAKER and ANDERSON, 1951), and (b) postautogamous death in *Paramecium* (KIMBALL, 1952). The extent to which chromosome structural change may contribute to these two forms of genetic damage has not yet been determined.

The Effect of Procedures Which are Likely to alter Oxygen Tension in the Cell

I have dwelt at some length on the influence of oxygen tension because the experimental results are in general more clear-cut than those obtained with other protective procedures, and tell us, I think, something important about the mechanism of radiobiologic damage.

The influence of hypoxia is so general that, in connection with any other procedure, we must first ask: "Will this alter the oxygen tension in the tissues?", since an affirmative answer will imply that to understand the whole effect, that part of it which is due to altered oxygen tension must be allowed for. For this reason it seems impossible at the moment to say whether many procedures affecting the delivery of oxygen to the tissues, namely, (a) bleeding, (b) ligature, (c) pentobarbitol, or nembutal anaesthesia, (d) drugs which restrict blood-flow, such as pitressin and acetyl-β-methylcholine, — all of which have been shown to give some degree of protection against roentgen radiation in experimental animals — have any specific action apart from their influence on oxygen tension. The problem is a complicated one, for we have seen that it is the oxygen tension at the instant of irradiation which is relevant, and this is a transient equilibrium condition determined by supply and consumption. For example, inhibition of respiration in a mass of tissue supplied with oxygen by diffusion would be expected to increase sensitivity. It will be recalled that tumour fragments irradiated in vitro were observed by CRABTREE (1934) to be more damaged by roentgen radiation when respiration was inhibited by cyanide. These observations have recently been confirmed by HALL (1951).

It is interesting that the protective power of the group of reducing sulphur compounds (such as sodium dithionate, $Na_2S_2O_4$), which HOLLAENDER and his collaborators (1951) showed to protect bacteria markedly against disinfection by roentgen radiation, has now been shown (BURNETT et al., 1952) by these authors to correlate completely with their influence on the oxygen tension of the medium in which the bacteria are irradiated.

In the same original experiments concerned with the disinfection of bacteria, it was shown that protection is also afforded by a group of substances including formate, succinate, pyruvate, and serine, but only if

the bacteria were incubated for a short period with these agents prior to irradiation. In recent experiments (STAPLETON *et al.*, 1952) it has been shown that incubation is ineffective if respiration is inhibited by cyanide, or in the case of succinate and pyruvate, by the addition of iodoacetate. A close correlation was observed between succinate protection and the measured inhibition of respiration. While the experiments are not as clear-cut as those with the sulphur compounds, in which the oxygen tension in the solution was recorded, we probably have here a demonstration that as an alternative to reducing the supply of oxygen, protection may be achieved by increasing metabolism in the presence of a limited oxygen supply.

The Influence of Immediate Pre-Treatment by Chemical Agents on Radiosensitivity

The fact that it appeared possible to trace many radiobiologic lesions to oxidative changes initiated by radicals prompted the study of the possible protective effect of strongly reducing amino acids, of which cysteine and glutathione have proved to be among those which are effective. In the course of a few years, many other compounds have been tested, notably by Professor BACQ and his collaborators (1951), who have discovered a particularly effective agent in β-mercaptoethylamine, for which the protective factor appears to be about 2.

As regards the protection afforded by cysteine and glutathione, I think we cannot as yet arrive at any conclusion regarding the mechanism involved, but the following points are noteworthy:

(1) The protective action of both these substances is rather general, one or the other, or both, not only increasing the survival of animals exposed to whole-body irradiation, but specifically reducing haematological damage, skin damage, the induction of cataract, the incorporation of C^{14} administered as labelled acetate into the DNA of young mice, radiation damage to tumour fragments and to thymocytes irradiated in vitro, inhibition of growth in onion roots, and chromosome damage in *Tradescantia* microspores.

(2) These substances might well act by reducing the concentration of dissolved oxygen in the cell, and in some cases do appear to act in this way, since, for example, FORSSBERG (1952) found cysteine markedly reduced the inhibition of growth of onion roots when irradiated aerobically, but not when irradiated anaerobically.

(3) FORSSBERG also found that cysteine protection resembled anaerobiosis in that no protection was afforded against α-ray damage.

(4) Unlike anaerobiosis, cysteine can protect against leukopoenia induced by nitrogen mustard.

(5) These agents exert their greatest protection if present in the cell during a period of the order of an hour before irradiation, as well as during irradiation. Later work has shown that they can also reduce radiation damage if administered after irradiation. In the case of glutathione, administration shortly after irradiation to heavily irradiated differentiated plant tissue permitted normal cell extension to take place (GORDON, STUTZ and WEBER (1950)). The dose was so large that in this case it may well have been that a proportion of all oxidisible SH groups in the cell were appreciably reduced by irradiation. PATT (1951) has shown that whole-body exposure of animals to doses of the order of 700 r of roentgen radiation does not diminish the number of oxidisible SH groups in several radiosensitive tissues, but nevertheless, cysteine can exert some protective action when added to a suspension of irradiated thymocytes immediately after irradiation. Fig. 3 is reproduced from the paper by PATT (1952).

The relation between effectiveness and time of administration shows a remarkable resemblance to some observations which I have made recently of the *increased* sensitivity induced in roots by carbon monoxide treatment.

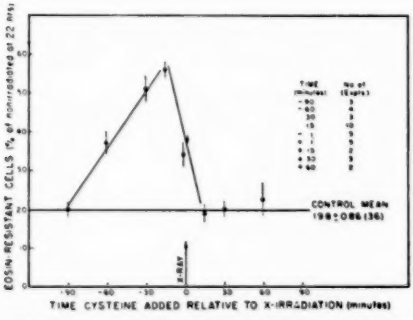

Diagram 3. Increased resistance of thymocytes to roentgen radiation as a result of treatment with cysteine before or after exposure to such radiation *in vitro*.

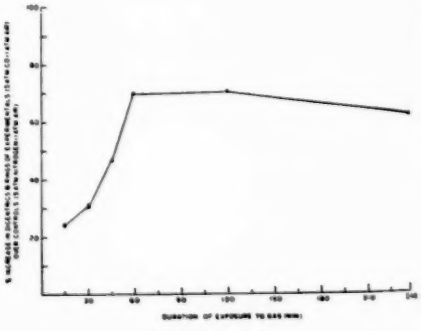

Diagram 4.

The Influence of Carbon Monoxide on Radiosensitivity

While almost all the many chemical treatments reported in the literature as affecting radiosensitivity (with the exception of cyanide, which has already been discussed) tend to protect the cell, KING, SCHNEIDERMAN and SAX (1952) have reported a substantial increase in the amount of chromosomal damage induced in *Tradescantia* microspores by a given dose of roentgen radiation when the influorescences are exposed for about

an hour prior to irradiation to a gas mixture consisting of one atmosphere of air and 5 atmospheres carbon monoxide. In control experiments, one atmosphere of air and 5 atmospheres of nitrogen was shown to be without effect on radiosensitivity. Figure 4, which is reproduced from the paper by KING, SCHNEIDERMAN and SAX (1952), shows the influence of carbon monoxide pre-treatments of various durations on the amount of chromosome damage induced by a given dose of roentgen radiation in *Tradescantia* microspores.

When I repeated the procedure used by these authors exactly, I found it to be without influence on the inhibition of growth of *Vicia* roots by roentgen radiation, within the limits of experimental accuracy, which were rather better than 10 %. This was surprising, since, as regards all other forms of radiobiological damage, *Vicia* meristems and *Tradescantia* microspores show a remarkable similarity. I therefore tried raising the pressure to 50/60 atmospheres. This pressure is not in itself injurious, provided it is applied slowly over a period of one or two minutes, and released slowly. At this pressure I obtained results very similar to those which SAX and his collaborators had obtained at 5 atmospheres pressure (a full account of these experiments will be published elsewhere). The principal results are shown diagrammatically in Diagrams 5 and 6.

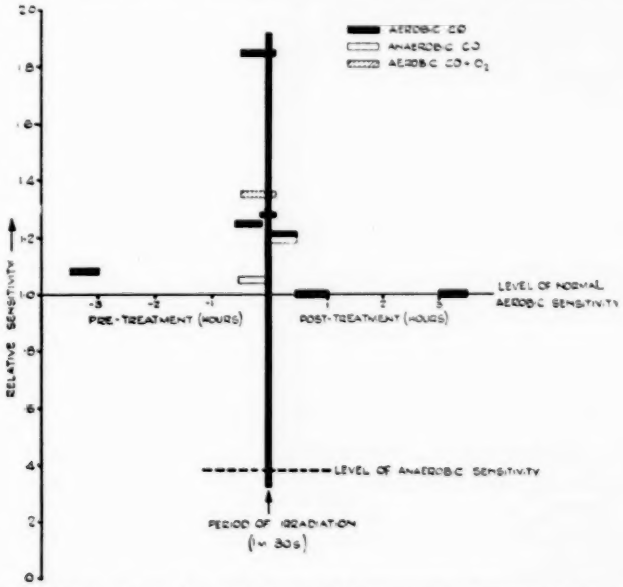

Diagram 5. Effect of aerobic and anaerobic monoxide treatment on sensitivity to roentgen radiation.

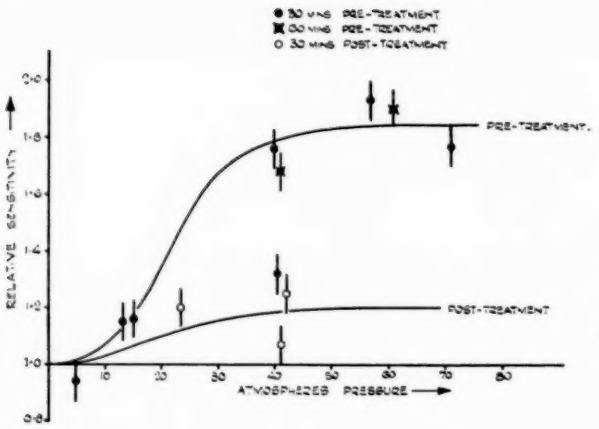

Diagram 6. Influence of pre-treatment & immediate post-treatment with carbon monoxide on sensitivity to roentgen radiation.

We may note:

(1) The greatest increase in sensitivity is obtained when the duration of the pre-treatment period is half to one hour and includes the irradiation period.

(2) This increased sensitivity is not due to the summation of two injuries, the one due to the carbon monoxide treatment, and the other due to the radiation, because the radiobiologic damage is unaffected by the same carbon monoxide treatment given three hours before or three hours after irradiation.

(3) In agreement with SAX, a treatment of a few minutes duration is much less effective than longer treatments, but little is gained by prolonging the treatment beyond about half an hour.

(4) Under optimum conditions the increase in sensitivity is about that observed by SAX, namely, a factor of 1·8.

(5) The effect of a small positive pressure of oxygen in addition to the large positive pressure of carbon monoxide is to reduce the effect of the carbon monoxide — and this despite the fact that oxygen alone increases radiobiological damage. A similar result was reported by SAX *et al.* This result tended to confirm their view that the influence of carbon monoxide on radiosensitivity resulted from inhibition of the cytochrome system of enzymes, with resultant reduction of molecular oxygen present in the cell to hydrogen peroxide by the flavoproteins. It was supposed that the effect of the hydrogen peroxide generated in this way augmented the effect of hydrogen peroxide or related radicals formed by the radiation.

(6) It is to be noted that if, after half an hour's pre-treatment with carbon monoxide, the roots are returned to ordinary air immediately

prior to irradiation and irradiated in air, most of the enhanced sensitivity is lost.

Our results provided two reasons for believing that the explanation is not wholly as given by KING, SCHNEIDERMAN and SAX:

(1) If the air is washed out, and roots are irradiated in carbon monoxide alone, then sensitivity is much the same as if the roots are irradiated in air. This is in marked contrast to the effect of replacing air by any of the inert gases, and implies that carbon monoxide can enter into the chemical reaction chain.[1] This is known to be the case from the experiments of FRICKE and HART (1938). At neutral and acid pH, and in the absence of oxygen, the irradiation of water containing dissolved carbon monoxide leads to the production of formaldehyde, which, besides being a highly toxic substance, has been shown to have marked mutagenic activity (AUERBACH 1951).

(2) In parallel experiments with neutron radiation carried out in collaboration with Mr. BOAG, a relatively insignificant increase in sensitivity was observed when the roots were irradiated at positive pressures of air and carbon monoxide, as shown in Diagram 7.

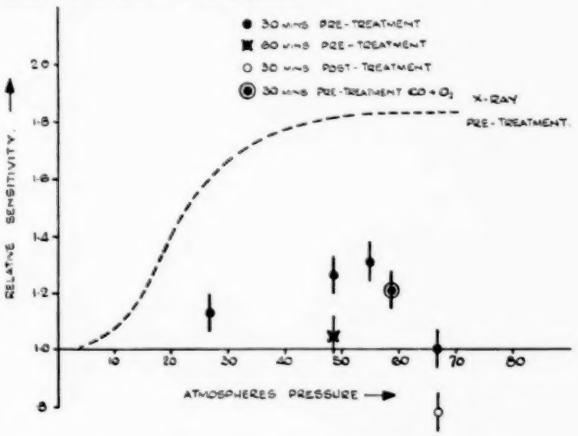

Diagram 7. Influence of pre-treatment and immediate post-treatment with carbon monoxide on sensitivity on neutron radiation.

The Development of Radiobiological Lesions — Post-Irradiation Treatments

It should not surprise us that radiobiological damage can be modified by a change in cell metabolism during the period immediately following irradiation. It is evident that the infinitesimal amount of energy deposited

[1] Note added in proof (18. 12. 53). In subsequent more intensive investigations, this observation could not be confirmed and is accordingly withdrawn.

in the cell by irradiation can do no more than initiate a sequence of changes which develop into a manifest lesion. The post-irradiation period is therefore a critical one, and it is logical to attempt to check the development of the injury.

Purely physical agents may either diminish or augment the ultimate damage resulting from irradiation. Thus, on the one hand, studies of photorestoration following exposure to UV radiation have shown that up to two-thirds of the damage induced by UV radiation may be prevented from becoming manifest by exposure to intense visible light shortly after irradiation. On the other hand, the fact that exposure to infra-red radiation (10,000 Å.U.) can increase the number of chromosome aberrations induced by roentgen radiation by as much as a factor of 3 shows that the ionizing particles produce changes in the chromosome at many points which do not normally develop into a complete break. It is interesting that in this case the latent injuries can be stored over periods as long as 96 hours, during which the cell is proceeding through its normal development, and that they may at any time be obliterated by a "heat shock" (SWANSON and YOST, 1951).

Through the courtesy of Dr. HOLLAENDER, I am able to mention some recent results to be reported shortly by STAPLETON, BILLEN and HOLLAENDER (1953), which are of considerable interest in relation to the development of damage induced by ionizing radiation. These authors have shown that after exposure to a given dose of roentgen radiation the proportion of bacteria capable of forming colonies, when incubated on a normal culture medium at 37° C., is greatly enhanced if the irradiated bacteria are first inbuted for 24 hours at some intermediate temperature. The intermediate temperature which is optimum for survival is characteristic of the bacterial strain. The exponential form of the inactivation curve is preserved — that is, as with photo-restoration following UV irradiation, the effectiveness of a given dose of radiation is reduced in a fixed proportion by a given post-irradiation treatment irrespective of the magnitude of the dose, up to eight times the 37 % inactivation dose. At the highest dose-level, the increased survivors far exceed the number of cells which have received a single lesion capable of inactivating the bacillus, and many of those which survive will have received several lesions each of which individually could have prevented the bacillus surviving to form a colony. That a fixed proportion of survivors, irrespective of dose, should, under such circumstances, be able to repair fully-developed lesions seems very improbable. It is more reasonable to assume that a proportion of those primary physical changes brought about by the individual electrons which would otherwise have developed into lethal injuries are prevented from so developing when the cell is incubated for a period at temperatures different from those at which it

normally grows. Preliminary experiments in which media other than nutrient broth were utilised indicated that irradiated cells are unable to recover (*i. e.*, the lesion is not prevented from developing) in an inorganic salts-glucose medium which will sustain growth and cell division in both irradiated and control cells. It is therefore suggested that a special nutritive material is required to secure the increased survival.

An interesting delayed interaction between a biologic structure and the irradiated medium has recently been discovered by Miss ALPER (1952) when studying the indirect inactivation of bacteriophage. It was observed that if virus which had been irradiated at high dilutions was allowed to stand at room temperature for some time before plating, the number of particles judged to be viable by their ability to form plaques when plated with sensitive bacteria was much smaller than if they were plated at once. It was suspected that the inactivation which took place after the end of irradiation might be due to hydrogen peroxide formed in the suspension medium, and in support of this view it was found that no delayed inactivation occurred when irradiation had been carried out in the absence of dissolved oxygen. In these respects the observations closely parallel the delayed changes observed when nucleic acid was irradiated in dilute solution by SPARROW (1946), TAYLOR, GREENSTEIN and HOLLAENDER (1947), and BUTLER (1950). In each case, however, this explanation ran into the difficulty that when samples of the virus or the nucleic acid were exposed to different concentrations of hydrogen peroxide, it was found that inactivation only occurred at much higher concentrations than those known to result from the given radiation exposure. This difficulty was resolved, at least in the case of the virus experiments, when Miss ALPER observed that the sensitivity of the irradiated virus to H_2O_2 was much greater than that of normal virus. (Diagram 8.) She has since shown that this is true of the bacteriophage T_3 as well as of S_{13}, and that both phages are more easily inactivated by ascorbic acid, as well as by hydrogen peroxide, if previously irradiated in dilute solution.

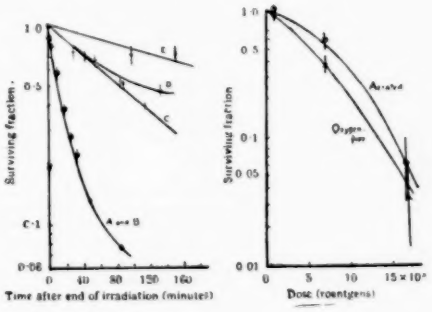

Diagram 8. The delayed inactivation of bacteriophage at high dilution resulting from exposure to roentgen radiation.

The circumstances of the experiment were such that a negligible proportion of the phage particles were directly ionized. In all cases we are therefore concerned with damage to the phage particles resulting from energy absorbed in the surrounding medium. It is seen that while some virus particles are rendered inviable by a primary injury which is complete within the period of irradiation, a much larger number may be rendered inviable by a two-step process involving interaction with hydrogen peroxide formed in the surrounding medium, if sufficient time has been allowed for this interaction to take place before the virus particles are plated with bacteria. The first step of the process is not dependent on dissolved oxygen, the second — the formation of peroxide — is, of course, oxygen dependent.

KIMBALL (1952) has recently observed that delayed division and non-genetic death in *Paramecia* are, under his conditions of irradiation, almost entirely due to the production of hydrogen peroxide in the surrounding medium, but in this case irradiation does not appear to have increased the sensitivity of the organisms to hydrogen peroxide appreciably.

Conclusion

When we pass from a consideration of the effects of radiation on cells to its effects on whole animals irradiated either wholly or in part, many new possibilities of interaction arise, both as between an injured organ and the capacity for restoration latent in the organism as a whole, or as between one organ and another. Several important examples of such interactions have been brought to light within the last few years, notably:

(1) Protection against whole-body exposure to roentgen radiation, achieved by shielding a mass of lymphoid tissue such as the spleen (JACOBSON, 1950 a and b), and the restoration resulting from bone marrow injections (CONGDON et al., 1952).

(2) The synergism between local and whole-body irradiation in causing regression of an implanted mouse lymphoma (HOLLCROFT, LORENZ and MATTHEWS, 1952).

(3) The work of LICK, KIRSCHBAUM and MIXER (1949), and later of KAPLAN (1950), which appears to demonstrate that ovarian tumours in mice, though initiated by radiation damage to the ovary, in fact develop in response to a disturbed endocrine balance which is secondary to degeneration of the ovarian tissue.

(4) FURTH's (1951) demonstration that pituitary tumours may result from total destruction of the thyroid by I^{131}. These tumours were at first only transplantable into thyroidectomised hosts, but one strain out of five was subsequently transplanted, grew and metastasised in a

normal host. They have so far remained under control, in that they atrophy under administration of thyroid hormone.

(5) KAPLAN'S (1952) work on the development of lymphoid tumours. Here it is of interest, from the point of view of radiation hazard, that (a) while tumour incidence increases with the dose, a given dose fractionated at four-day intervals is more effective than a single exposure, and (b) that keeping the dose to each part of the body constant, the tumour incidence depends markedly on how much of the body is exposed at any one time, exposure in two halves, for example, giving 5 % incidence as against 52 % incidence for a simultaneous exposure, which is the optimum for tumour induction.

It is beyond the scope of this paper, as well as beyond my competence, to discuss the significance of these experimental observations. Acute exposure to radiation places the body under stress, severely damages the haematopoietic system, lowers resistance to infection, and does many other kinds of damage. The therapy of such exposures is likely for some time to constitute a medical rather than a radiobiologic problem. On the other hand, the radiation hazard which is incurred occupationally, or to which a population at large may be exposed through the peaceful applications of atomic energy, is essentially a low-level premeditated exposure, and it is reasonable to expect that through the study of the radiobiology of the primary lesion we may avoid exposure at times when the body is especially sensitive and perhaps even find means of maintaining the body in a condition of increased resistance without interfering with normal life.

SUMMARY

Some experimental studies of the effect of pre- and post-irradiation treatments of cells on biological damage resulting from exposure to ionizing radiation are reviewed. The influence of oxygen tension on damage induced by roentgen radiation is shown to follow a strikingly similar pattern in the case of five different biological responses in plant and insect cells, whereas its influence on α-ray and neutron damage is relatively slight. The enhancement of roentgen damage by pre-treatment with a gaseous atmosphere of air and carbon monoxide, reported by KING, SCHNEIDERMAN and SAX for *Tradescantia* microspores has been confirmed for *Vicia* roots by the author, but only by the use of large positive pressures of carbon monoxide. The effect is relatively slight, or non-existent, in the case of neutron radiation.

ZUSAMMENFASSUNG

Einige experimentelle Studien über die Wirkung von Behandlungen — vor und nach der Bestrahlung — von Zellen mit biologischen Schäden, die auf Grund von Exponierung mit jonisierenden Strahlen entstanden sind, werden beschrieben. Der Einfluss der Sauerstoffspannung auf durch Röntgenstrahlen entstandene Schäden folgt einem völlig gleichförmigen Schema in dem Fall von 5 verschiedenen biologischen Antworten

bei wachsenden und ruhenden Zellen, dagegen ist der Einfluss auf Schäden durch α-Strahlen und Neutronen relativ gering. Die Verstärkung von Röntgenschäden durch Vorbehandlung mit einer gasförmigen Atmosphäre von Luft und Kohlenmonoxyd, über die KING, SCHNEIDERMAN und SAX für *Tradescantiamikrosporen* berichten, ist für *Viciawurzeln* vom Verf. bestätigt worden, jedoch nur bei Benutzung von hohem positiven Druck des Kohlenmonoxydes. Der Effekt ist in Fällen von Neutronbestrahlung relativ gering oder unbefindlich.

RÉSUMÉ

L'auteur passe en revue quelques études expérimentales concernant les effets de traitements avant et après irradiation de cellules sur les troubles biologiques résultant de l'exposition à des radiations ionisantes. Il est démontré que l'influence de la tension d'oxygène sur les troubles causés par les rayons roentgen obéit à des lois remarquablement semblables pour cinq réponses biologiques différentes sur des cellules de végétaux et d'insectes, alors que son influence sur les troubles dus aux rayons alpha ou aux neutrons est relativement faible. L'aggravation des troubles dus aus rayons roentgen après un traitement préalable par une atmosphère d'air et d'oxyde de carbone, signalée par KING, SCHNEIDERMAN et SAX pour les microspores de *Tradescantia* a été confirmée pour les racines de *Vicia* par l'auteur, mais seulement avec des pressions fortement positives d'oxyde de carbone. L'effet est relativement faible on nul dans le cas d'irradiation par des neutrons.

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SPONTANEOUS AND INDUCED CELL RESTORATIONS AFTER TREATMENTS WITH IONIZING AND NON-IONIZING RADIATIONS

by

Raymond Latarjet

The restoration of irradiated cells has both a theoretical and a practical interest. Theoretically, it contributes to a better understanding of certain processes which take place during the development of the radiolesion, and it may even indicate the nature of chemical or biologic reactions. From the practical point of view, this restoration shows that the development of lesions in cells, tissues or organisms, which a former irradiation has doomed to permanent injury, may be counteracted. In this respect, the problem seems more important than that of protection, although it will always be more difficult to solve.

In the present paper we shall steadily keep in mind the general observations given in the preceding statement by LATARJET and GRAY, namely: 1) the general scheme for the development of any observable change; 2) the definition of the term 'restoration', tantamount to a successful post-treatment, which carries "no particular connotation regarding the mechanism by which the development of the lesion is modified".

I. Natural Restorations

That the restoration phenomenon is possible has been demonstrated by the existence of natural cases, both after ionizing and non-ionizing radiations. A beautiful example is given by DELAPORTE's observations on aggregated bacteria (1951).

A full-grown culture of E. coli, strain B, in broth, was irradiated with a dose of UV which left 10^{-2} colony forming cells. An aliquot was seeded on complete agar and incubated for one hour at 37°. Microscopic observation showed that by that time some cells had started growing

(not dividing; division had a lag phase of about 4 hours); the others had not. It showed also that some cells were isolated on the agar, whereas the others were in clumps of 2 to 37 cells. Statistical numerations disclosed the following facts:

a) After incubation in the dark, the probability that an isolated cell grows is 7 times less than that of any individual cell belonging to a clump. The probability for the latter is independent of the number of cells within the clump, being the same for a 2-cell as for a 30-cell clump. Therefore, contact between cells favours the growth of each of them by a factor of 7.

b) Within a given clump, all the cells were in the same growing situation. All or none of them grew. As soon as one cell starts growing, it induces the other cells of the clump to grow. This induction requires a close contact; there is no induction at some distance. The most probable explanation for this phenomenon is that a growing cell can transfer to other cells, through simple contact, a substance capable of restoring their capacity to grow.

Answers to the following two questions would be of considerable interest: 1) What is the nature of the substances which secure such restorations by diffusion from cell to cell? 2) Does such a phenomenon occur in tissues where cells are closely connected? It is unfortunate that the same experiments have not yet been repeated after roentgen irradiation.

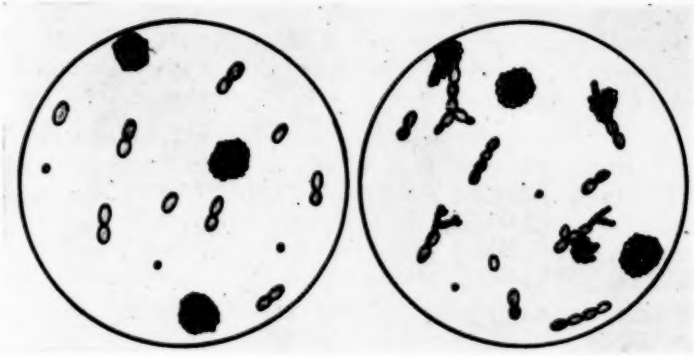
Another case of natural restoration, this time after ionizing radiations, is illustrated by diploid yeasts. When irradiated with roentgen, α-, or β-rays, then incubated on nutrient medium, they display the classical figures described by LACASSAGNE and HOLWECK (1930). If one compares these figures to those from haploid yeasts under the same conditions, a striking difference appears (LATARJET and EPHRUSSI, 1949). In irradiated haploids, the figures are limited to:

— normal colonies arising from uninjured cells;
— single cells, normal in size or enlarged (immediate death);
— pairs of enlarged cells (delayed death after one division).

These figures are final and do not change in the course of prolonged incubation. Haploid yeasts do not recover spontaneously (Fig. 1).

In the diploids, on the other hand, one observes besides the preceding figures, numerous recovery figures which develop in the course of incubation. On chains of giant cells apparently doomed to die, a cell of normal appearance often arises and from which a normal colony develops.

This type of natural restoration is linked to diploidy.



S. Cerevisiae

Haploid Diploid

Fig. 1. Aspect of colonies and injured cells of Saccharomyces cerevisiae after roentgen irradiation.

II. 3 Types

It is obvious that restoration, in the broad sense in which it is being considered in this paper, may be secured, according to the circumstances, by many different processes which may act at different stages during the development of the lesion. For clarification, these processes will be classified according to 3 types.

1°) *Restoration by reversion*

A reaction A ⟶ B, of the chain initiated by the radiation, is reversed by the restoring process which either induces the reaction B ⟶ A, or destroys B, thus cutting the chain.

The simplest possible case of reversion is the deactivation of an activated molecule with return to its normal state. In fact, many primary radiochemical reactions consist in lifting a molecule (a nucleoprotein, for example) from its normal level A to an activated state A*. This jump absorbs a quantum w of radiant energy:

$$A + w \longrightarrow A^*$$

A* is a metastable state which eventually may reverse to A. It does not express itself by the observable lesion unless it falls, by another exothermic path, down to another stable state B, the probability for this type of deactivation being different and possibly higher than that leading back to A (Diagram 1). In such a situation, restoration can be

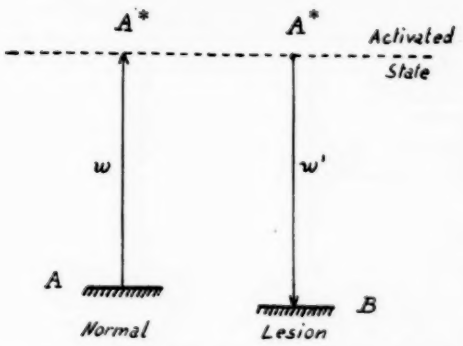


Diagram 1. Molecular change following activation.

secured by deactivating A* to A. The life span of the activated state represents the period during which this restoration remains possible.

Such a phenomenon is well established in periodic structures, such as crystals and high polymers, where deactivation may be induced by radiation of long wave length or by heating (quenching of phosphorescence). It is very likely that pseudo-cristalline structures such as nucleoproteins — in which electrons may migrate and be trapped in the same fashion as in crystals (MOTT and GURNEY 1940, MÖGLICH and ROMPE 1941), and where peroxides have the same effect as in high polymers (CONWAY and BUTLER 1952) — may undergo such deactivations under light or under temperature shocks. In these cases, the restoring process deals with the very first radiochemical reactions.

Some other reversions may occur, dealing with later steps, by the knocking out of intermediate active compounds rather than by the induction of reverse reactions. For example, catalase restores division in some microorganisms treated by UV radiation by destroying certain intracellular organic peroxydes produced by the irradiation (Section IV).

2°) *Restoration by recovery*

The lesion often results from the destruction or inactivation of a cellular constituent. Making up this deficiency will restore, by supplementing the cell with this constituent, either from without, *i. e.* from an external supply, or from within by favouring its synthesis under an active form by the cell itself.

The Delaporte effect is a good example of supply from without: a cell, whose capacity to grow has been inhibited by UV-irradiation, recovers this capacity when it comes into close contact with a growing

cell, *i. e.* (probably) when it receives from the latter an unknown growth-restoring factor.

Recovery of the capacity to synthesize an active compound may be illustrated by multiplicity-reactivation in a virus-bacterium complex (LURIA 1947): a bacterium, infected with one UV-inactivated phage particle, cannot grow active virus (this is the definition of virus inactivation); but, in the case of certain viruses such as coli phage T_2, a bacterium infected with several UV-inactivated particles, displays a high probability of growing active virus.

In more general terms, one must consider that the process involved in the chain of reactions leading to the lesion, interferes and competes with the cellular metabolisms, some of which, although more or less disturbed by irradiation, may secure different kinds of 'physiologic' recovery. Any factor which influences this competition against the lesion will behave as a restoring agent (see Section III).

3°) *Restoration by superimposed injury*

In order to develop, the chain of reactions leading to the lesion may require some normal metabolite from the cell. For example, one step may be:

$$\begin{matrix} B \longrightarrow C \\ + X \end{matrix}$$

X being a cell constituent. If one eliminates X (superimposed injury), without killing the cell, one cuts the chain and prevents the lesion from developing. We shall see later (Section V) that some restorations induced by visible light are likely to proceed this way.

III. Restorations Induced by Temperature Changes

As previously mentioned (section II, 1°), a temperature shock can reverse some earlier activated states back to the initial situation, thus erasing the primary effect produced by the absorption of radiant energy. As an example, this mechanism has been considered in order to explain the counteracting effect of a temperature shock on the additivity of infra-red and roentgen rays in the production of chromosome aberrations in Tradescantia (SWANSON and YOST 1951). In these experiments, the aberrations produced by roentgen rays were doubled in frequency by infra-red rays, given prior to, also following the former, although not in themselves capable of producing such aberrations. It is assumed that both roentgen and infra-red rays induce activated states in the genetic material of the cell, probably in nucleoprotein molecules. These states have no morphologic expression; they persist for long periods of time

at room temperature. If, between the two radiations, irrespective of their order, the cells are maintained at 48° C for 30 seconds, the potentiating action of the IR is prevented from being expressed in the form of an increase in the frequency of aberrations. These activated states are removed by high temperatures when once induced. According to SWANSON and YOST, in chromosomes of Tradescantia these states become highly unstable, hence undetectable, if induced at temperatures above 23° C.

Temperature changes may act more generally by favouring physiologic recoveries (Section II 2°). If one considers the competition which takes place within the cell between the reactions leading to the lesion and some cellular metabolisms, one appreciates that temperature will induce recoveries in so far as it affects differently the speeds of the reactions called into play, and in the sense of this difference. When there is no such difference, no temperature recovery takes place; when temperature favours the lesion reactions, some restoration will be produced by cooling; on the other hand, when recovery reactions are favoured, restoration will follow heating. One may therefore expect temperature effects in both directions, according to the lesion considered. Experience agrees with this expectation both with ionizing and non-ionizing radiations.

1°) *Cooling*

STRANGEWAYS and FELL (1927) were first to demonstrate restoration by cooling when they showed that degenerative modifications produced by roentgen rays in chicken embryos were lessened and sometimes stopped if embryos were kept for 24 hr at 5° C after irradiation. They concluded that placing the organism for some time in a resting stage gave the tissues the possibility of repairing the lesions.

COOK (1939) confirmed this observation on Ascaris eggs submitted to a roentgen dose of 5,000 r. The controls incubated at 25° C immediately after irradiation gave 2 p. 100 normal embryos. The other groups were kept at 5° C for increasing periods, then incubated. This exposure to cold increased progressively the proportion of normals: 4 p. 100 after a period of 1 week; 15 p. 100 after 4 weeks; 45 p. 100 after 8 weeks, after which no further recovery took place.

A stay at 5° also induces recovery in diploid yeasts treated with either UV or roentgen rays (LATARJET 1943). The frequency of killed cells, as deduced from colony counts after incubation, decreased by a factor of 2 to 4 within a few days.

MILLER and PLOUGH (1952) recently observed another case of restoration by cooling after UV, when inducing a biochemical back mutation in Salmonella typhimurium. The irradiated cells were kept for 48 hours at 0°, 19° or 37° C, then incubated at 37° C on a minimal medium which

selected the mutants. The numbers of induced mutations finally showing up were as follows:

Storage temperature during 48 hr	U. V. dose (ergs mm^{-2})			
	0	300	600	1,200
0° C	4	109	118	139
19° C	3	163	206	289
37° C	4	228	45	208

2°) *Heating*

The opposite effect may occur. According to ANDERSON (1951), the survival of UV treated bacteria from E. coli strain B, as measured by colony forming cells, increases when the temperature of incubation on complete agar is raised from 30° to 40° C, with a 20 fold increase between 30° and 37° C, and another 20 fold between 37° and 40° C. No such effect was observed on strain B/r.

Working also with strain B, STEIN and MEUTZNER (1951) found the highest survival after 2 hours' storage at 44.5° C immediately following the UV irradiation. If, before applying this treatment, one incubates the irradiated cells at 37° C, their heat restorability remains unchanged for 1 hour, then drops to half its value after 2 hours, and finally disappears after 4 hours. This means that the cellular reactions involved in this type of restoration are already completed after 4 hours at 37° C.

A comparison of heat restorations in strains B and B/r show how complex the situation is. B/r is a radiation resistant mutant isolated from B by WITKIN (1946) and extensively used for its high photorestorability. When incubated at 37° C, B/r is about 10 times as resistant as B (in order to obtain the same killing in both strains, one must deliver about 10 times more radiation to B/r than to B). But when incubated at 44° C after irradiation, both strains display the same resistance, that of B having increased twentyfold, and that of B/r twofold only (HARM and STEIN 1952). It seems that the mutation from B to B/r alters the temperature sensitive system which accounts for the heat restoration in B.

B/r displays an even more complex behaviour when treated with roentgen rays, then incubated for 24 hours at different temperatures from 6° to 37° C, and finally incubated at 37° C (STAPLETON et al. 1952).[1] The survival increases regularly when the temperature is raised from 6° to 18° C, then decreases symmetrically when the temperature is raised further on, survivals being equal and at their lowest value at

[1] See Dr. Gray's paper.

6° and 37° C. That this temperature effect depends on cell metabolisms is clearly demonstrated by its taking place on complete nutrient medium only. On a minimal synthetic medium which just secures growth and division, the temperature effect disappears.

IV. Restoration Induced by Catalase

It was stated in section II, 1°, that restoration could be brought about by the destruction of some intermediate active compound. As far as I know, the only clear cut case of such type of chemical restoration is presently exemplified by the effect of catalase in some microorganisms.

MONOD et al. (1949) observed that the survival of UV treated E. coli K-12, estimated by colony counts, increased with the number of cells plated. The dead bacteria contributed some restoring factor which the authors identified as catalase. This restoring effect (C. R.) was favoured by the administration of visible light, insufficient in itself for giving a notable restoration. Since this discovery, studies carried on in my laboratory have disclosed the following facts (LATARJET and CALDAS 1952, MILETIĆ and MORENNE 1952, and unpublished data).

Table 1

P. 13: 8×10^6 bacteria per cm³. U. V: 1,250 ergs mm⁻²
Assays: 0.1 cm³ per plate

	Number of colonies	
	without catalase	with catalase
Dark	13	804
Light	37	4,764

C. R. in P. pyocyanea P. 13.

Table 2

K. 12 S: 5×10^6 bacteria per cm³. U. V.: 2,200 ergs mm⁻²
Assays: 0.1 cm³ per plate

	Number of colonies	
	without catalase	with catalase
Dark	1	2,140
Light	8	3,050

C. R. in E. coli K. 12 S.

1°) Tables 1 and 2 describe two typical experiments, one in which a little visible light greatly enhances the restoring effect, and one in which catalase alone is about as active as when associated with light. In most cases, the maximum CR yields about a 1,000 fold increase in colony counts. It takes place when high doses of UV have been delivered, leaving from 10^{-5} to 10^{-4} normal survivors. Smaller and higher doses lead to lesser CR. This restoration depends on strict but still undefined physiologic conditions of the cells, both before and after irradiation.

2°) The three non-lysogenic bacteria tested so far (E. coli strains B and B/r, B. dysenteriae Flexner Y6R) give no or very little CR. On the other hand, all four lysogenic strains tested (B. Megatherium 899, E. coli K-12, P. pyocyanea strains 13 and 13 (8)) are restored. CR seems linked to lysogenicity. Moreover, when losing its lysogenicity, a lysogenic strain retains its restorability by catalase. For example, non-lysogenic strains Megatherium Mox and Coli K-12S, derived from the preceding 899 and K-12 respectively, are as efficiently restored by catalase as their lysogenic parents.

3°) Catalase acts or fixes itself upon the bacteria within a few minutes; the restored cells start dividing at the same time as the normal survivors. When irradiated coli are stored at 37° on nutrient medium, their restorability lasts for about 2 hours, then drops and disappears around the 4th hour. (The same figure has been encountered in heat-recovery of UV treated coli (Section III, 2°).) This means that catalase acts on substances which either are relatively stable, or are formed within the cell rather late (about 2 hours) after the irradiation.

Finally, CR is not produced after roentgen irradiation.

4°) If we now look at the origin of catalase activity, we must point out that other organic molecules, such as haemoglobin and chlorophyll, with a similar structure but no activity on peroxides, have no restoring power. On the contrary, the enzyme peroxidase extracted from horse-radish (kindly supplied by Dr. B. C. SAUNDERS) is as effective as catalase. If one treats the same irradiated sample with either catalase or peroxidase, one obtains such similar numbers of restored cells that one may assume that both enzymes restore the same cells. This means that they both act in the same way.

Moreover, experiments carried on samples of progressively heated catalase tested for decomposition of hydrogen peroxide and for restoring capacity, show that both actions run parallel. When heating increases, they start dropping and finally disappear together.

This link between restoring capacity and enzymatic activity of catalase strongly suggests that restoration is due to the decomposition of peroxides which are known as lethal and mutagenic agents on micro-

organisms (DICKEY et al. 1949), and as active intermediates produced by UV in some organic media (WYSS et al. 1950).

From protection experiments, it seems that the cellular compounds involved in CR are organic peroxides of the RO — OH type rather than H_2O_2. Pyruvate, an acceptor to H_2O_2, which it decomposes, but not to organic peroxides, protects bacteria against UV but does not restore them (THOMPSON et al., 1951). Since in this case protection is very probably due to decomposition of H_2O_2, this observation shows that hydrogen peroxide is rapidly produced by the irradiation of the cell protoplasm, and is then quickly engaged into further reactions. On the other hand, catalase, which reacts with both H_2O_2 and organic peroxides of the RO — OH type, both protects and restores. When added to lysogenic as well as non-lysogenic strains before UV irradiation, it behaves the same as pyruvate, yielding the same increase in survival. This protection therefore probably deals with early formed and short-lived H_2O_2. When added after UV (or before, in such an excess that some free enzyme remains available once irradiation and protection are achieved), then, in lysogenic strains only, it produces a restoration by dealing with compounds which, because of their sensitivity to catalase and insensitivity to pyruvate, strongly appear as organic peroxides. Since at 37° C restorability lasts several hours, and since H_2O_2 disappears early, these peroxides, which could be formed by H_2O_2 itself, must have long lives.

Let us consider at this point the chemical effect of catalase. One might assume that catalase acts by reason of its so-called catalatic power, by decomposing peroxides, according to the equation:

$$2\ RO — OH \longrightarrow 2\ R — OH + O_2$$

If this were true, one should be able to explain why the nascent O_2 does not initiate new lethal oxidation processes, and why peroxidase, which is not endowed by this catalatic power, restores as effectively as catalase.

On the other hand, catalase and peroxidase share the property of catalyzing the oxidation of certain organic substrates by nascent peroxides. Since, in these reactions, the peroxide comes from a former oxidation, such processes are called coupled oxidations — these have been demonstrated both with catalase (KEILIN and HARTREE 1945) and with peroxidase (SAUNDERS and WATSON 1950). We may now consider that both enzymes achieve their restoring action by initiating coupled oxidations of certain cellular substrates, thus diverting the radio-lesion process from its normal pathway.

Diagram 2 summarizes these considerations. It should not be forgotten that the restorable process illustrated in this figure represents a mode

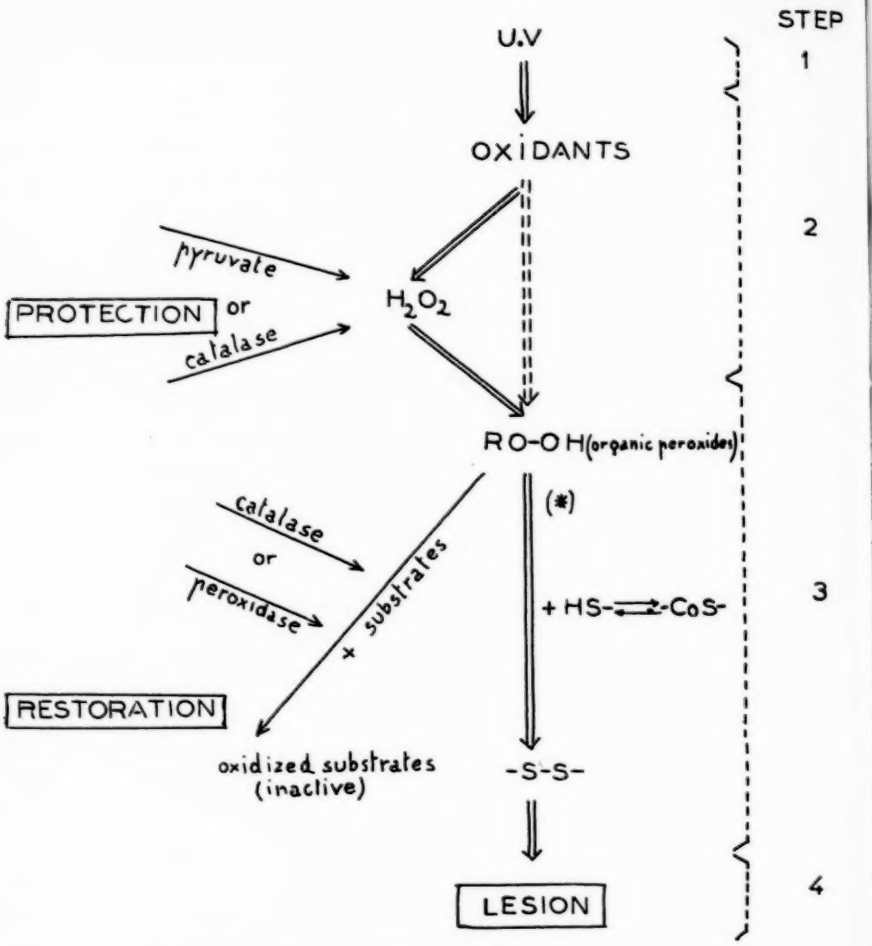


Diagram 2. Scheme of the process.

Double arrows refer to normal process leading to the lesion; simple arrows refer to induced protection and restoration.

Steps refer to those considered by LATARJET and GRAY in the hereabove statement.

(*) This reaction is given here only as an example selected among many possible ones. The equilibrium between HS and CoS keeps cupper in the cuprous state and protects HS against oxidations catalysed by cupper, whereas — S — S — can no longer bind metals (ALBERT 1952).

of killing of minor importance if compared to the total killing due to UV-light. Never more than 1 out of 100 irradiated cells is restored by catalase. It is almost certain that, according to the hypothesis that NOVICK and SZILARD (1949) formulated after photo-restoration experiments, the lethal action of UV follows two different main pathways. Besides the catalase sensitive process, one must consider that UV, mainly absorbed in proteins and nucleoproteins, produces, without the intervention of peroxides, more or less marked and reversible denaturations which follow the establishment of excited states in these molecules themselves. (The overwhelming importance of this process is supported by the similarity between the spectral absorption curve of nucleoproteins and the spectral curve of monochromatic killing efficiency.) In a similar way, a progressive heating produces discrete denaturations, some of which appear accessible to chemical restorations. For example, assuming that certain denaturations may be due to the opening of CH bonds in proteins, CHRISTOPHERSEN and KAUFMANN (1952) succeeded in restoring heated yeasts by treatments with phenol and glycocolle the charge of which contributes to the re-establishment of the CH bonds.

V. Restoration by Light

Since KELNER's discovery (1949), it has been observed that all lesions due to interactions between cellular nucleoproteins and UV light are, up to a certain extent, restored by radiations of the spectral range 3,500—5,000 A, under the condition that illumination is not given too late after UV. By "up to a certain extent", it is understood that the appearance of the lesion is entirely prevented in a certain fraction only of the treated population, as though, according to a preceding remark, the individual lesion was determined either by a restorable process or, more often, by a non-restorable one.

The chemical mechanism of P. R. will probably remain unknown as long as we do not know with high accuracy the spectral curves of monochromatic efficiency which will reveal the chromophores put into play.

One can hardly assume that light achieves restoration by inducing the reverse reaction B $\longrightarrow$ A from a reaction A $\longrightarrow$ B initiated by UV. If it were so, extracellular viruses inactivated by UV should be restorable by light whilst in this naked state, and this P. R. should proceed independently from temperature. This is not the case, and the term photoreversal which appears sometimes in the literature should be dropped as implicating a wrong mechanism.

According to DULBECCO (1949), a bacteriophage inactivated by UV may be photorestored only when adsorbed by its sensitive host, a peculiarity which is also displayed by plant viruses (BAWDEN and KLECZ-

KOWSKY (1952) and might be general to all viruses. BLUM et al. (1951) found a similar situation in their study of irradiated sea urchin egg and sperm, the sperm corresponding to the virus, the egg to the host-cell, and the fertilized egg to the infected cell: sperm inactivated by UV is not restored by light, but becomes restorable as soon as it has entered the egg.

UV-inactivation of viruses and sperm deals with nucleoproteins (HNP), as it is shown by the spectral curves of monochromatic efficiency, and is proved by the fact that when a phage infects its host, the NP fraction only enters the cell, leaving the protein bag, when it exists, outside (HERSHEY and CHASE 1952). Therefore, P. R. is clearly relevant to NP synthesis. It provides the cell for the capacity of synthesizing some NP which otherwise would not be synthesized because it enters the cell in a damaged state, unsuitable to synthesis. In other words, light transforms UV-inactivated NP into active NP by reason of a particular action on the cell-NP complex. Neither illumination of the cell nor illumination of the inactive NP prior to infection, secure this transformation the essence of which remains undeciphered.

This change in the complex is temperature sensitive (BOWEN 1952), which can be explained either by the existence of dark reactions in the process induced by light, or by the interference of this process with a normal chemical equilibrium of the cell. Temperature sensitivity of P. R. has been encountered in several other instances.

As already pointed out, the restoring effect of light on inactivated NP is a rare phenomenon rather than a general one. In order to yield restorable NP, inactivation must proceed in a progressive and gentle way. NP inactivated by heat or by ionizing radiations are not restored by light, and, even in the case of UV, the individual quantum of action of which is low (4 to 5 eV), only a minority of biologic units within an irradiated population are left in a restorable condition.

That light does not restore after ionizing radiations is not always true if the term restoration is understood in the general sense which has been defined at the beginning of this paper. In fact, induction by roentgen rays of virus synthesis in lysogenic bacteria can be erased by light, and this phenomenon illustrates what I called "restoration by superimposed injury" (section II, 3°). The experiments were carried out as follows (LATARJET 1951) on cultures of lysogenic B. megatherium in the logarithmic growing stage in broth. Light does not alter this growth of normal bacteria (Diagram 3, curve A). If, at time zero, the culture is irradiated with 30,000 r of roentgen rays, its growth rate remains unchanged for a while, but most cells are 'induced': the virus, which they hereditarily carry in a dormant form, is turned by the irradiation

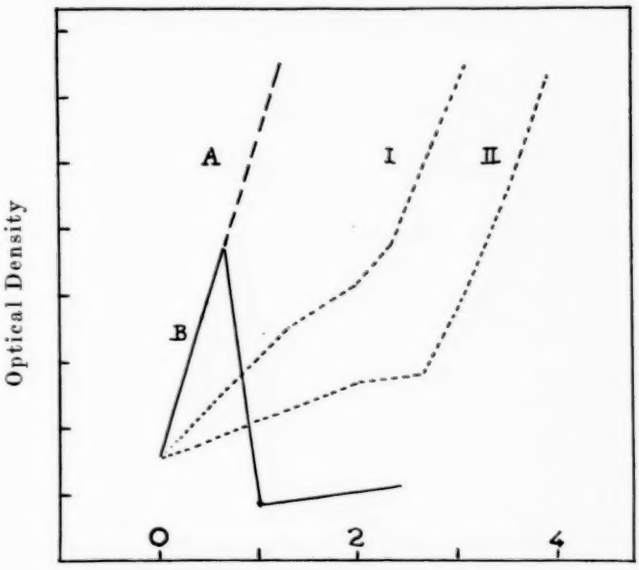


Diagram 3. Photorestoration of induction by roentgen rays in lysogenic B. megatherium. A. Normal growth. B. Induction by roentgen rays. I—II. Roentgen rays + light.

into an active one which initiates the synthesis of numerous and active virus particles.

Within about 40 minutes these cells pass through 2 divisions, then they lyse and liberate in the medium the particles which they have completed by that time. The suspension clears up; its optical density drops abruptly (curve B).

Now, if one illuminates the culture early after the inducing roentgen ray shock, one stops synthesis of the virus and prevents lysis of the cells; cell growth proceeds, then divisions take place, and the culture finally reaches the same normal situation as it would, had no irradiation been given (curves I and II). In this case, light does not reverse the primary effects due to roentgen rays; it cuts the chains that these effects have opened, and prevents them from reaching their two main expressions: synthesis of active virus and cell lysis. If one considers the population in its entirety and these expressions as the final lesion produced

by roentgen rays, one may say that light given after roentgen rays restores the population.

Experimental curves I and II (curve II corresponds to a dose of light twice as high as that of curve I) disclose that light, which did not alter the growth of untreated cells, markedly slowed down that of induced cells. (The same decrease in growth rate, yielding the same type of curves, has been observed on the same material during its restoration by fasting (LWOFF et al. 1950). This similarity supports the idea of a process by superimposed lesion.) According to LATARJET and MILETIĆ (1952), illumination of organic media such as broth produces substances, probably organic peroxides, which, practically inactive on normal cells, display a high toxicity on induced and virus infected bacteria, as though the first stages of intracellular virus growth sensitized the cells to these substances. Strong illumination therefore produces this unexpected toxic effect (superimposed injury) on the induced cells. Although complete analysis of this phenomenon is still lacking, we may assume that some of these cells are killed and will neither yield active virus nor bacterial colonies; the others, less deeply altered by light can no longer synthesize the virus; they keep growing at a slower rate — as shown by the curves —, then divide into cells henceforth endowed with normal growth rate and capacity to divide.

SUMMARY

A critical survey of the restoration phenomenon in irradiated cells is presented. It appears that several different processes, as the case may be, are brought into play. The author distinguishes between three general processes: R. by reversion, R. by recovery, and R. by superimposed injury. Each process is illustrated by experimental results obtained by means of temperature changes, chemicals, or visible light.

ZUSAMMENFASSUNG

Kritische Übersicht der Restaurationsphenomene der bestrahlten Zellen. Es scheint dass verschiedene Mechanismen je nach den Fallen einwirken. Der Verfasser unterscheidet drei allgemeine Prozesse: R. durch Reversion, R. durch Wiederherstellung, und R. durch zugefügte Beschädigung. Jedes Prozess ist durch experimentale Ergebnisse, erhalten durch Temperaturmodifikationen, durch chemische Substanzen, oder durch sichtbares Licht, dargestellt.

RÉSUMÉ

Revue critique du phénomène de restauration des cellules irradiées. Il apparaît que divers processus sont mis en jeu selon les cas. L'auteur distingue trois processus généraux: R. par réversion, R. par récupération, et R. par lésion surajoutée. Chacun est illustré par des résultats expérimentaux obtenus au moyen de changements de température, de produits chimiques, ou de lumière visible.

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THE EFFECTS OF IONIZING RADIATIONS IN BARLEY

by

L. Ehrenberg and *Å. Gustafsson*

When analyzing the biologic effects of ionizing agents we have to search for material which permits a complete study of (1) the immediate effects, in cytoplasm and nucleus, direct or indirect, on properties like viscosity, respiration, chromosome breakage, (2) later effects like seedling growth, survival up to the stage of reproduction, diploid and haploid sterility, and (3) the second generation properties of mutation frequency and mutant viability. Certain agricultural plants, for example barley, wheat, oats, peas, afford excellent material for such a complete analysis. In the following we concentrate on the barley plant.

This report deals with a short summary of some recent results, obtained by the cooperation of numerous scientists working on cytoplasmic and chromosomal effects of radiations and aiming at the production of useful mutants in agricultural and horticultural plants. EHRENBERG considers some results relevant to the abovementioned points 1 and 2 and GUSTAFSSON certain results in connection with points 2 and 3.

The embryos of dormant seeds of barley contain about 10 % of water, most of which, of course, is chemically bound to proteins, etc. In spite of the low water concentration in the embryos we have to consider an indirect action of the roentgen radiation, since hydrogen sulfide, when present in various amounts of the atmosphere (1—50 %), exerts a marked protective action. This protection is probably similar to that normally exerted by sulfhydryl compounds in waterous tissue. However, no influence of the oxygen pressure on the radiation sensitivity can be noticed, if, two hours before irradiation, the air surrounding the dormant seeds is exchanged for nitrogen or oxygen. A possible explanation is that oxygen does not partake in the radiation-induced chemical reactions of dormant seeds. Since a small effect of oxygen is observed when its pressure is changed 24 hours before irradiation, a slow penetration of

oxygen into dormant seeds or a slow change of the physiologic state in the seeds due to the altered oxygen pressure have to be considered.

Germinating seeds, *i. e.* seeds pre-soaked in water for 24 hours, are very sensitive to changes in the oxygen pressure 30 minutes before irradiation: Pure oxygen increases the radiation damage, whereas nitrogen and hydrogen have a distinct protective influence. The importance of the physiologic state on the radiation sensitivity is demonstrated by the fact that anesthetics protect against radiation. The expression 'physiologic state' is here used for factors associated with irritability and metabolic rate. Propene shows a protective action both with regard to germinating and dormant seeds. Ether given in high, anesthetizing concentrations protects the germinating seeds from damage, in contrast to low concentrations (apparently increasing the irritability) which augment the damage. Of course, a chemical protection through the double bond of the propene molecule (*cf.* FORSSBERG's results on the protective action of fumaric and maleic acids) or an unshared electron pair of the ether molecule is not excluded, but the fact that magnesium ions, too, which do not protect by chemical means, decrease the radiation damage, makes the conclusion plausible that anesthesia counteracts the radiation damage.

Since oxygen partakes in the radiation induced reactions, it seems to us important to elucidate what effect all the treatments influencing radiation sensitivity exert on the respiration rate and, consequently, on the intracellular oxygen concentration.

The action of fast neutrons (circa 3 MeV), which have a higher specific ionization than roentgen rays, seems to be fundamentally different from that of the latter. H_2S shows no protective action on dormant seeds, and there is no distinct effect of the oxygen pressure on the radiosensitivity of germinating seeds. With regard to the lethalizing and growth-inhibiting actions of the two kinds of radiation at least partly different processes seem to be influenced. In the case of roentgen treatment, most of the seeds which are not immediately killed recover from the damage through some sort of elimination of injured cells. After neutron treatment, on the other hand, the seeds germinate and grow normally for a few days and then suddenly die, probably at a point of time when cell divisions become important for the development of the plant. In the mature plants, grown in the field, there are also striking differences in behaviour after roentgen and neutron treatments. Roentgen ray plants rarely show a lower average spike fertility than 50 per cent. Neutron plants, on the other hand, produce drastically decreased average fertilities down to 20 or 30 per cent.

Lethal and semilethal, as well as other viability-decreasing mutations are easily induced by ionizing radiations. In barley, one or two com-

pletely viable, or high-productive, mutations appear in one thousand induced changes. The rest are more or less harmful to the organism in the diploid state. However, it has been recently found that there are a series of highly complex interreactions with regard to viability, characteristic of the individual mutations. Certain homozygous mutations increase viability under hard competition, others increase productivity when competition is relaxed. Very few types can be immediately used in practice. Some mutations, lethal when homozygous, increase viability when heterozygous. Some again decrease viability both in the heterozygous and homozygous state. It is possible that in barley every mutation, so far studied, whether morphologic or physiologic in its action, drastic or minor, viable or lethal, has a reaction mode of its own, changing with the type of environment and the genotypical milieu.

With the increasing application of ionizing radiations for peaceful purposes, or in warfare, there will no doubt be an accumulation of deleterious changes also in man, in addition to the changes spontaneously arising, if precautions are not extremely rigorous. Proportionally seen there will be a considerable increase of long chromosome deficiencies spreading in the population. This will more rapidly lead to conditions of homozygous lethality than under natural conditions, since lethal factors will more easily find their mates, owing to the destruction of long sequences of gene material. In addition, chromosome rearrangements, like inversions and translocations, are readily produced by ionizing agents, but not for instance by ultraviolet radiation. Chromosome breakage is, to all appearances, facilitated by clusters of ionizations. Densely ionizing agents, like neutrons and α-rays, are therefore highly effective in chromosome breakage. In barley they cause special end-results not observed with roentgen rays and probably nowhere found spontaneously. Two-point breakages, resulting in long deficiencies and translocations, will therefore be more frequent with densely ionizing radiations. Finally, there are distinct indications (discovered by KAPLAN, KNAPP, GUSTAFSSON and co-workers), that different types of mutations will appear as the result of different treatments previous to irradiation. Off-hand, induced and spontaneous mutations cannot be considered as essentially alike or occurring in unalterable proportions.

The barley plant shows that productive mutations may be induced by roentgen rays, and possibly also by neutrons. In agricultural plants the 99 per cent or more of radiation-induced damage is easily eliminated. In man this intentional elimination of deleterious changes cannot easily be applied. The repeated warnings of H. J. MULLER against radiation-induced damage appear to be fully justified.

SUMMARY

The results are reported of work upon cytoplasmic and chromosomal effects aimed at the production of useful mutants in barley. These are considered from the points of view of the immediate effects, the later effects and the second generation properties of mutation frequency and mutant viability.

ZUSAMMENFASSUNG

Die Ergebnisse einer Arbeit über zytoplasmatische und chromosomale Wirkungen werden mitgeteilt. Die Arbeit bezweckte nützliche Kornmutanten zu produzieren. Die Resultate werden vom Gesichtspunkt der unmittelbaren Wirkungen, der Spätwirkungen und der Eigenschaften der zweiten Generation bezüglich der Mutationshäufigkeit und der Variabilität der Mutanten betrachtet.

RÉSUMÉ

Les auteurs présentent les résultats d'un travail sur des effets cytoplasmiques et chromosomiques destinés à produire des mutants utiles de l'orge. Ces résultats concernent les effets immédiats, les effets retardés et les propriétés de fréquence des mutations et de viabilité des mutants de la seconde génération.

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FROM THE DONNER LABORATORY, UNIVERSITY OF CALIFORNIA, BERKELEY, CALIFORNIA, U. S. A.

REMARKS ON THE RADIOSENSITIVITY OF UNICELLULAR ORGANISMS

by

Cornelius A. Tobias[1]

During the present meeting we have heard interesting discussions on the different aspects of the biologic effects of radiation; particular emphasis was laid on the mechanism of the radiation effect as mediated by chemical reactions and on the availability of different ways of protecting living cells and tissues by means of externally applied chemical substances. I wish to emphasize in this brief review some of the inheritable characteristics of radiosensitivity.

For the past several years efforts have been made in various laboratories omit to find the site of action of radiations in the cells and to explain the shape of the survival curves which have been obtained. Some investigators have attempted to differentiate the defects depending on the ploidy of the cells. I should like to mention FRÖIER, GELIN and GUSTAFSSON, LATARJET and EPHRUSSI. In the United States during the past few years opportunity for experimentation with the different ploidies was presented through the isolation by LINDEGREN of haploid and diploid Saccharomyces cerevisiae. LINDEGREN kindly put some of his strains at the disposal of RAYMOND ZIRKLE and myself, and at the University of Chicago and at the University of California in Berkeley systematic work was begun to study radiation sensitivity as a function of ploidy. Preliminary results have been described and more complete information is in press (ZIRKLE and TOBIAS, 1954).

The inhibition of cell division was studied not only as a function of ploidy but also at various different specific ionizations. The rate of energy loss of the particles varied from 6×10^6 ev/gm cm^2 (high energy deuterons and roentgen rays) to 2×10^9 ev/gm cm^2 (low energy alpha particles). The work is being extended now to even higher specific ionizations by the use of 120 Mev six times ionized carbon particles

[1] The author's work was supported by the U. S. Atomic Energy Commission.

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Cornelius A. Tobias[1]

During the present meeting we have heard interesting discussions on the different aspects of the biologic effects of radiation; particular emphasis was laid on the mechanism of the radiation effect as mediated by chemical reactions and on the availability of different ways of protecting living cells and tissues by means of externally applied chemical substances. I wish to emphasize in this brief review some of the inheritable characteristics of radiosensitivity.

For the past several years efforts have been made in various laboratories omit to find the site of action of radiations in the cells and to explain the shape of the survival curves which have been obtained. Some investigators have attempted to differentiate the defects depending on the ploidy of the cells. I should like to mention FRÖIER, GELIN and GUSTAFSSON, LATARJET and EPHRUSSI. In the United States during the past few years opportunity for experimentation with the different ploidies was presented through the isolation by LINDEGREN of haploid and diploid Saccharomyces cerevisiae. LINDEGREN kindly put some of his strains at the disposal of RAYMOND ZIRKLE and myself, and at the University of Chicago and at the University of California in Berkeley systematic work was begun to study radiation sensitivity as a function of ploidy. Preliminary results have been described and more complete information is in press (ZIRKLE and TOBIAS, 1954).

The inhibition of cell division was studied not only as a function of ploidy but also at various different specific ionizations. The rate of energy loss of the particles varied from 6×10^6 ev/gm cm^2 (high energy deuterons and roentgen rays) to 2×10^9 ev/gm cm^2 (low energy alpha particles). The work is being extended now to even higher specific ionizations by the use of 120 Mev six times ionized carbon particles

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produced in the cyclotron (BIRGE and SAYEG). Subsequently the inhibition of cell division by radiation was also investigated under anaerobic conditions (BIRGE and TOBIAS). All these different experimental conditions were imposed on the same microorganisms in an effort to find out the actual physical mechanism of the radiation effects. The criterion for accepting the mechanism would be that all the different survival curves obtained under different environmental conditions would have to be more or less quantitatively in agreement with the model. By this analysis we thought to connect the gap between the so called 'hit theory' so successfully used in the early days of radiobiology and the intermediate action theory that is overwhelmingly favored in biochemical experiments on enzyme suspensions in vitro. The analysis of the data obtained indicates the following:

The division of the haploid yeast cells and their ability to form colonies can be inhibited by a single deuteron or alpha particle penetrating into the cell. The alpha particle, however, is more effective than the deuteron in causing the inhibition. Furthermore, anaerobism gives some protection to the haploid cells by increasing the mean lethal dose. However, in this case it is still true that a single particle can cause inhibition of the cell division.

Diploid cells can only be inhibited if more than one particle crosses the cell, except for a small percentage of cases where a single particle might be effective. The diploid-deuteron survival curve also changes when the radiation happens in the anaerobic state. Both haploid and diploid survival curves are quantitatively accounted for if one assumes that the lethal action is genetic in nature and that the haploid cell can be injured by a single particle in any one of several ways (MORTIMER and TOBIAS). The place where an injury might happen was named the 'essential genetic site'. The simple model outlined here gives the number of sites as 25 ± 15. However, with more refined models this number might be considerably modified in the future.

The diploid cells are inhibited in cell division mainly if a pair of essential genetic sites are inactivated, that is if the damage done is homozygous. It also appears that the probability of inactivating each site is about the same.

The diploid cells are about five times as resistant to radiation as haploid cells. However, when the ploidy becomes higher the cells become more sensitive again. These were studied by MORTIMER in the Berkeley group and he found that in order to account for the increased sensitivity at higher ploidies, it is necessary to assume that single particles become more effective in causing dominant lethal effects.

It is not possible to account for the survival curves by assuming that the particles go directly through the sensitive sites. It is likely that

in a large proportion of the cases the action of the radiation is indirect and that there are intermediate chemical substances responsible for the action. The migration theory of biologic effect has been outlined (TOBIAS, WIJSMAN). The nature of the sensitive sites seems to have more biologic significance than physical, that is, they seem to be connected with biologic processes which are essential to cell division and somewhat independent from each other. As to how large the sites are in space and in how many places they are vulnerable to ionization or intermediates, we are not prepared to say at the present time.

It is possible to obtain mutations to radiation sensitivity by selecting individual pre-irradiated diploid cells and growing colonies from them (TOBIAS and STEPKA). Many of these cells have unpaired or recessive lethal damage inflicted by the radiation and this is exhibited by an inheritable increased radiosensitivity as compared to normal diploids. However, a mechanism for recovery also exists — if the cells are allowed to grow for a prolonged period of time, the radiosensitivity of the colonies returns to normal.

The radiation resistance of haploid and diploid yeast cells depends on the state of cell division. Preliminary evidence is available that sometime during cell division the resistance to radiation injury is as much as 7 times the resistance in the resting stage (BEAM, et al.). Haploid and diploid cells obtained from different sources seem to have somewhat different radiation sensitivity on the same medium even though the general mechanism of inhibition appears to be the same in each of these strains. On this basis it should be possible to carry out genetic experiments and to isolate genes linked to radiation sensitivity.

This brief review of work on radiobiologic work on microorganisms is given at this discussion to indicate that there are other approaches besides the biochemical one in our efforts to understand the mechanism of radiation effects and in providing agents or methods for protection against radiation.

SUMMARY

The communication consists of a brief review of some of the inheritable characteristics of radio-sensitivity. Radiation sensitivity as a function of ploidy is discussed in detail and developed as an approach other than the biochemical one to the problems pertaining to the mechanism of radiation effects and the questions of protection.

ZUSAMMENFASSUNG

Die Mitteilung besteht aus einem kurzen Überblick über die erblichen Charakteristika der Radiosensibilität. Die Strahlenempfindlichkeit als eine Funktion der Chromosomenzahl wird eingehend besprochen und es wird versucht, anders als biochemisch an die Probleme heranzugehen, die für den Mechanismus der Strahlenwirkungen und für die Strahlenschutzfragen gelten.

RÉSUMÉ

Cette communication consiste en une brève étude de quelques caractéristiques héréditaires de la radiosensibilité. La radiosensibilité en tant que fonction de la ploidie est étudiée en détail et présentée comme une hypothèse de travail, différente de l'hypothèse biochimique, pour l'étude des problèmes relatifs au mécanisme des effets des radiations et aux questions de protection.

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